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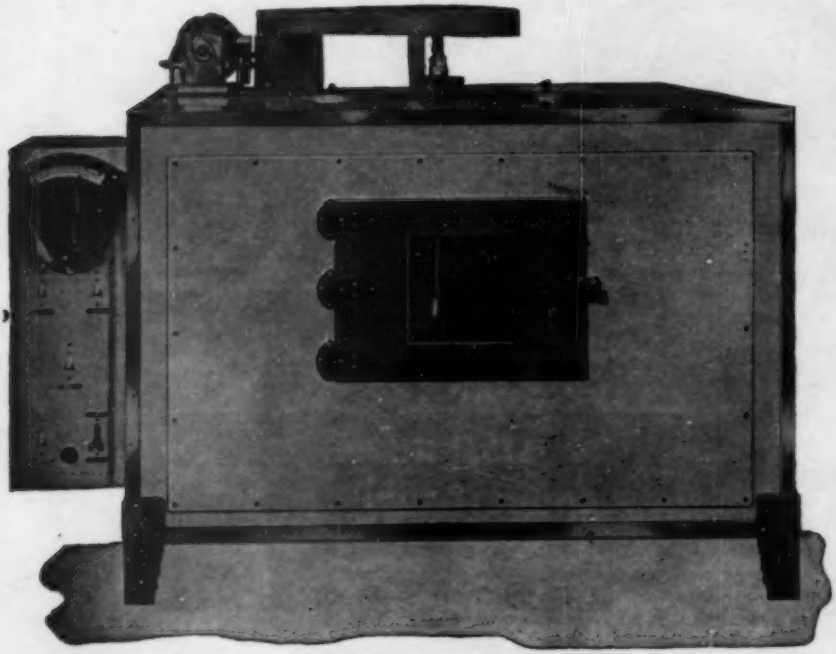

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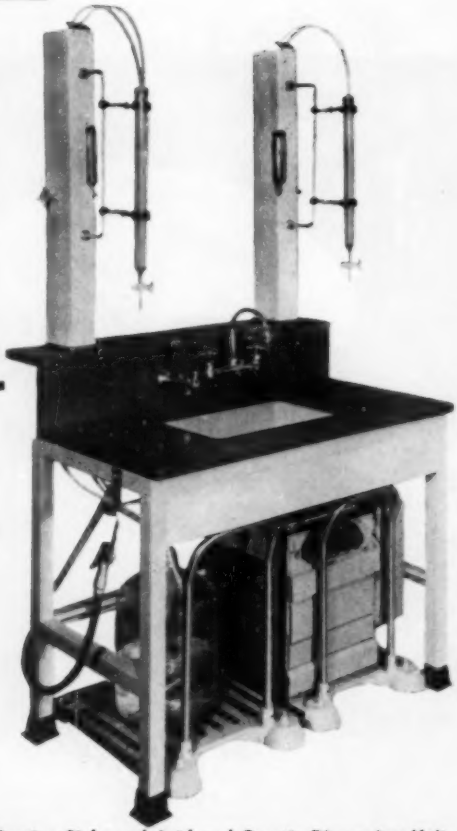

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CEREAL CHEMISTRY

VOL. 37 MAY, 1960 NO. 3

EFFECT OF THE RELATIVE QUANTITY OF FLOUR FRACTIONS ON CAKE QUALITY[1]

D. H. DONELSON AND J. T. WILSON[2]

ABSTRACT

An unbleached, commercially milled 48% extraction soft wheat flour was fractionated by a batter method into water-solubles, gluten, starch tailings, and prime starch. Flour blends using various proportions of the fractions were prepared and bleached. White layer cakes were baked using a lean formulation. A batter-mixing schedule incorporating a preliminary doughing step was necessary in order to obtain layers comparable in volume and internal structure to cakes baked from the parent flour.

The baking quality of these reconstituted flours appeared to be strongly influenced by their composition. By means of a central composite statistical design, a multiple regression equation was derived relating layer volume to the relative proportions of the fractions making up the flours. Because of interaction, responses of fractions varied significantly at different concentrations of the fractions. It was found that, in general, the water-soluble fraction tended to decrease cake volume, although not greatly, and that tailings had a marked effect in increasing volume and improving internal appearance. Small changes of concentration of gluten or of prime starch above or below the normal amounts had little effect on volume, but much greater or smaller than normal concentrations of either resulted in much smaller cakes.

It was concluded that the relative proportions or balance of flour components conditioned the contribution to cake structure of each component and had a significant effect upon the quality of the cake.

At this laboratory one of the general approaches to the investigation of cake flour quality has been by baking cakes from reconstituted flours. Such flours are produced by combination of four flour components: water-solubles, gluten, starch tailings, and prime starch, ob-

[1] Manuscript received January 27, 1959. Contribution from the Soft Wheat Quality Laboratory, Ohio Agricultural Experiment Station, Wooster, Ohio. A report of the NC-30 research project of the North Central States Soft Wheat Technical Committee. Presented in part at 43rd annual meeting, AACC, Cincinnati, Ohio, April 1958.

[2] Research Staff, Department of Agronomy, Ohio Agricultural Experiment Station, Wooster, Ohio.

tained by a process of fractionation.

Sollars (11), by means of a reconstitution technique, investigated the effect of chlorine bleaching on flour fractions in cakes and cookies. The properties of baking-powder biscuits were studied by interchanging flour components from two sources of flour and in two compositions by Zaehringer *et al.* (13). Yamazaki studied the effect of starch tailings on cookie diameter (12). This paper reports an investigation of the variation of white layer cake quality with changes in the proportions of the component flour fractions. In this case, the measure of quality was chosen to be cake volume, with due consideration of internal appearance and contour. Since the effect of a given fraction might depend upon the specific levels of the other components, it was necessary to utilize a multifactor experimental design.

Materials and Methods

Flour. An unbleached 48% extraction commercially milled soft wheat cake flour was used as the source of all the flour fractions. The percentages of the components and their protein content as found by fractionation are shown in Table I.

TABLE I

PERCENTAGE COMPOSITION AND PROTEIN CONTENT OF FLOUR FRACTIONS

MATERIAL	COMPOSITION [a]	PROTEIN CONTENT [b]
	%	%
Unbleached flour	100.0	7.6
Water-solubles	3.6	22.2
Gluten	10.0	60.7
Starch tailings	10.7	3.53
Prime starch	75.7	0.35

[a] Adjusted to 14% moisture basis; fraction percentages adjusted to 100%.
[b] 14% moisture basis. Protein = N × 5.7.

Flour Fractionation. Sixty-five pounds of the unbleached flour was fractionated by a batter method adapted from the process reported by Adams (1). A batter, or slack dough, was formed by mixing 500 g. flour and 380 ml. distilled water in a Hobart C-10 laboratory cake mixer with a paddle at medium speed to optimum gluten development (about 10 minutes). This dough was then whipped for 2 minutes at low speed with 950 ml. distilled water to form a slurry in which the gluten was aggregated as chunks. All the mix was run on a vibrating 25 standard silk bolting-cloth screen. Gluten was collected from the surface of the screen as large pieces and washed by hand-kneading with three 50-ml. portions of water. The washings were added back to

the screen. The filtrate from the screening was centrifuged for 15 minutes at 1,800 r.p.m. in an International centrifuge. The supernatant, which contained the water-soluble fraction, was decanted and the layered residue was separated with a knife into starch tailings (top layer) and prime starch (bottom layer). A small residue of highly hydrated material was retained on the screen and this was added to the tailings fraction. Each fraction was then frozen to the inner walls of 4,000-ml. jars, lyophilized, and ground at the fine setting of a Hobart coffee mill. This method of fractionation gave a 97.5% recovery of materials and reasonably complete separation. The procedure has been used successfully to fractionate several low-protein soft wheat flours. The amount of water required in the batter-forming step, and the batter mixing time, are both rather critical and must be adjusted according to the requirements of each flour.

Flour Reconstitution. Flour blends were prepared by mixing the dry components in the proportions needed for each treatment combination of the experimental design. The blends were hydrated to approximately the moisture content of the original flour (12%), by exposure on stainless-steel trays to a high humidity atmosphere in a closed cabinet. Each flour was then bleached with chlorine gas in laboratory equipment to a pH of 4.8, the optimum level found for the original flour.

Cake Baking. The cake formula used in testing these cake flours was a modification of the lean white layer formula developed by Kissell (9). This formula was developed as a test of the baking capacity of cake flours and contains only flour, water, shortening, sugar, and baking powder. The omission of milk and eggs eliminates all structure-forming elements except the flour, and the very high sugar content places additional stress upon the flour. The method of testing is very sensitive to the liquid-carrying capacity of the flour, to the extent of bleaching, and to varietal differences. The formula is summarized in Table II.

The cake batter mixing schedule for reconstituted flours required a preliminary dough mixing operation, as described by Sollars (10). The flour and about 75% of the water required were mixed in a pin-type bread mixer to optimum gluten development. The dough was then transferred to a Hobart C-100 cake mixer, and shortening, blended sugar and baking powder, and additional water were added. These ingredients were mixed 0.5 minute at low speed, then 1.5 minute at medium speed. The balance of the water required was added to the mass and the batter mixed 0.5 minute at low speed, then 1.5 minute at medium speed.

TABLE II
LEAN WHITE LAYER CAKE FORMULA (MODIFIED)

INGREDIENT	QUANTITY	FLOUR BASIS
	g	%
Flour	150.0 (14% m.b.)	100.0
Sugar (Bakers' Special)[a]	195.0	130.0
Shortening (high ratio, emulsified)	41.8	27.9
Baking powder (double action)	7.05	4.7
	ml	
Water (to optimum level)	145.5–163.5	97–109

[a] In baking cakes from whole flours, 212 ml. of 69% sucrose solution are used to supply the sugar and part of the water.

The preliminary dough step seemed to be necessary because the reconstituted flours were merely mechanical mixtures of ground fractions. Apparently an intimate recombination of components was required, and attempts to obtain cakes directly from reconstituted blends by standard full formula or by the regular one-step lean formula were unsuccessful. Attempts to obtain cakes directly from flours produced by lyophilizing a dough made up from the reconstituted blends (12) were also unsuccessful. The batters appeared curdled, the shortening was not well dispersed, and the baked products were of very low volume and relatively structureless. The two-step dough and batter method apparently permitted the organization of a protein matrix strong enough to disperse the shortening and yield a smooth batter, and to provide a basic matrix within which the final cake structure developed, without being so well defined and cohesive as to give a breadlike structure. Reconstituted cakes baked during a 2-year period with this two-step method were comparable in volume and contour to those baked from the corresponding whole flours. Internal structure, flour bleach optima, optimum liquid level, etc., were similar to those of cakes baked from the whole flour, although the over-all texture was somewhat coarser and cell-wall thickness was slightly greater. It seems likely that the gluten development in the batters of reconstituted flours is greater than that in batters using whole flours, where a dough step is not necessary.

An indication of the importance of the dough step in forming an organized or structured batter can be gained from the fact that variation of dough mixing times and liquid level both affected final cake volume. In preliminary bakes, any combination of absorption and mixing time that did not permit optimum gluten development in a firm dough was associated with lowered cake volume. With respect to total liquid level, the lean formulation is rather sensitive. For this study trial bakes determined the amount of water that would yield

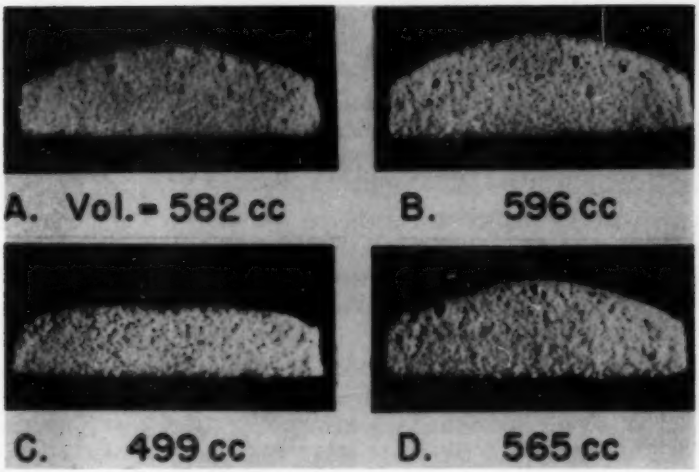

Fig. 1. Cross-section of lean-formula cakes. A, baked with whole flour; B, C, and D, baked with reconstituted flours, treatments No. 1, 3, and 10 respectively.

the maximum volume and optimum top contour (rounded, with this formula) for each treatment combination. Most bakes required 103% liquid level, the optimum level of the whole flour, although absorption throughout the whole series ranged from 97 to 109% for different flour compositions.

Each batter was scaled at 240 g. into two 6-in. bottom-lined, greased-wall steel cake pans and baked at 375°F. for 21 minutes. Cake volumes were measured by seed displacement and taken as the mean between two layers. The internal structure and contour scores were determined by the system of Kissell (9). Generally, the internal appearance was slightly coarser than that of cakes baked from the bleached whole flour and of cakes baked from a commercial control flour. Figure 1 shows sections of cakes baked from the whole flour and from several treatments of the reconstituted flours.

Experimental Design. A preliminary baking study suggested that observed differences in cake volumes might be ascribed to interactions among flour components, so an experimental procedure in which the amount of one fraction at a time was varied, with the other three held at fixed levels, would yield rather restricted information. The effect of one fraction at fixed levels of the other three might be rather different from its effect at other levels of the rest of the components, a quite familiar situation in chemical synthesis. Indeed, holding all fractions but one at fixed levels introduces a logical *non sequitur.* Any

alteration made to the composition of a flour, either by recombining fractions in different proportions or by enriching a whole flour by addition of a fraction, is bound by the condition that the sum of the percentages of the components must equal 100%. For example, if a series of baking tests is made using flour enriched with gluten, the gluten additions effect not only an increase in the percentage of gluten, but a decrease in the percentage of the other flour components. Any result ascribed to the change in gluten concentration is confounded with change possibly due to decrease in concentration of the other flour components. It is questionable whether experimentation in which one factor at a time is varied yields any straightforward result independent of this confounding. For a study such as this, where the aim of the experiment is to follow the effect on cake quality of alterations of the proportions of flour components, which is in part just an attempt to investigate whether the confounding mentioned is important or not, this constraint becomes a major concern.

It was hypothesized that, in part, cake quality is a function of the relative concentrations of flour components used. That is, two cakes baked from the same flour fractions might be of rather different quality if the proportions of fractions used in each were different. It appeared possible to do a series of test bakes using flour fractions from a single flour recombined in a variety of proportions, in order to obtain some rational estimate whether the concentration aspect of these quality differences was significant, apart from any question concerning the inherent quality of flour components.

It seemed appropriate to attempt to fit a multiple regression equation as an estimate of the quality-concentration response. Within the concentration constraint mentioned, the independent variables, whether expressed as amounts, percentages, or ratios of flour fractions, were capable of being varied continuously and could be taken at required levels with essentially only random error, in order to obtain the quality response. The response selected as the measure of quality was layer volume, since this is straightforwardly amenable to quantitative treatment, does not entail many arbitrary judgments on the part of the experimenter, and is not therefore biased to the extent that cake-scoring procedures might be. Indeed, layer volume is a fairly good measure of quality, both commercially and in the laboratory, since most rating methods give it great weight, and with the lean-formula baking test, volume is a rather good index of flour quality. It was felt that use of this criterion, in conjunction with scores as a supplement, would give a fairly satisfactory measure of baking quality, so long as the flour fractions used in the treatments were all from the same

source.

We can postulate a functional relationship:

$$y = \psi(\xi_1, \xi_2, \xi_3, \xi_4),$$

where y is cake volume and the ξ_i are the amounts of water solubles, gluten, tailings, and prime starch. With all other baking conditions, such as amount of reconstituted flour and other batter ingredients, equal for each treatment, or else arranged by preliminary tests of factors such as liquid level and dough mixing time so as to yield the best layer obtainable for each treatment, the functional relation derived should be an acceptable approximation. It was thought that a satisfactory first approximation to the form of this functional relation would be a polynomial of second order in the four independent variables. The mixed terms would allow some estimate of interaction. However, the subsidiary condition of this experiment, that the sum of the percentages of the four components must be equal to 100%, would lead to indeterminate normal equations in the estimation of the coefficients of the polynomial if the percentages were used directly as the independent variables, because of linear dependence of these variables. In order to obtain a set of variables such that each could be changed independently of the others, ratios of the percentages of flour fractions were selected for a transformation of the regression equation. These ratios were:

x_1 = water solubles/(gluten + tailings + starch)
x_2 + gluten/(tailings + starch)
x_3 = tailings/starch

Each of these ratios could be varied independently of the other two, but specification of all three uniquely determined the amounts of all four components, and vice versa. Admittedly, such a transformation makes interpretation of data rather awkward, but it affords a solution to the mathematical problem involved. An approximation to the transformed functional relation between cake volume and composition was made by fitting a second-order polynomial in the three variables; i.e., the familiar Taylor series expansion in three variables with all terms beyond second order considered as a negligible remainder, $R(x_i)$:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + R(x_i)$$

The cross product terms would afford some estimate of interaction, the remainder term would show the adequacy of the second-order approximation, and statistical analysis would indicate the degree and location of significance of various factors.

Such a mathematical apparatus is, of course, a common way to set

up a multiple regression problem. The major statistical and experimental problem of fitting a regression equation is the *rational selection* of treatment combinations to use; i.e., the design, on the basis of compromise among the desired goodness of fit of the derived equation, the amount of experimental material available, the ability to solve the coefficient matrix, etc. A complete factorial design, for example, in the three variables at a minimum of three levels of each would require 27 treatments, and such a design might be considered as the traditional way of selecting treatment combinations to test. But, as pointed out by Hader *et al.* (8), when a polynomial approximation is used, more precise estimates of the coefficients are available by use of designs other than factorial.

A design of the Box-Wilson multiple response surface type was chosen as the best available means of gaining a test of the hypothesis and to obtain a measure of the relative effect of the four flour fractions. The treatment combinations were selected to form a central composite second-order design, comprising 22 treatments, each replicated. These comprised five levels of each variable. Hackler *et al.* (7), Hader *et al.* (8), and Box (2) discuss the advantage of this type of design for multilevel multifactor evaluation; Cochran and Cox (5) present typical forms, and Box (3,4) and Davies (6) present several discussions concerning interpretation of experimental data. In general, the basic design provides a rational way to estimate the joint effect of several factors simultaneously, with a minimum number of experimental tests, to solve the coefficient matrices straightforwardly and to obtain statistical data conveniently.

When the experiment was planned, it was decided to select ranges of flour composition that seemed at the time likely to give the maximum information, although some of the compositions were rather abnormal. The ranges of flour component percentages were as follows: 1 to 7%, water-solubles; 5 to 17%, gluten; 3 to 20%, starch tailings; and 65 to 82%, prime starch. Such a wide range of variation would show whether cake volume did indeed depend to any appreciable extent on the composition of the reconstituted flour and might show how each factor contributed to cake volume, although for a given number of treatments there might be only a fair fit of the regression equation. In case one should require a better approximation, it would be possible to take narrower ranges in an extension of the experiment, or increase the number of treatments or add additional terms to the fitted polynomial expression. The center point of the Box-Wilson design was taken as the reconstituted flour composition:

4.1% water-solubles; 11.4% gluten; 12.0% tailings; and 72.5% prime starch; a composition rather typical of soft red winter patent flours. Of the points taken for treatment combinations, this center point was closest to the proportions actually found by fractionation of the flour (Table I).

The range for each ratio was divided into five equally spaced levels, in the absence of any reason for scaling otherwise. To facilitate calculation of the coefficients in the matrix solution, the ratios were coded as follows:

$$X_1 = \frac{-0.0430 + x_1}{0.0165}$$

$$X_2 = \frac{-0.135 + x_2}{0.040}$$

$$X_3 = \frac{-0.166 + x_3}{0.063}$$

Table III lists the compositions, coded values of the ratios, cake volumes, and scores of all treatments. The volumes and scores were

TABLE III

TREATMENT COMBINATIONS WITH CORRESPONDING FLOUR COMPOSITIONS, CAKE VOLUMES, AND SCORES

Treatment No.	Variable Coded Value			Flour Composition [a]				Cake Volume Average	Cake Score Average
	X_1	X_2	X_3	Water Solubles	Gluten	Starch Tailings	Prime Starch		
				%	%	%	%	cc	
1	0	0	0	4.1	11.4	12.0	72.5	596	5.0
2	0	0	0	4.1	11.4	12.0	72.5	587	6.5
3	−1	−1	−1	2.6	8.5	8.3	80.6	499	5.3
4	−1	−1	+1	2.6	8.5	16.6	72.3	595	7.5
5	−1	+1	−1	2.6	14.5	7.7	75.2	566	5.8
6	−1	+1	+1	2.6	14.5	15.5	67.4	585	7.0
7	+1	−1	−1	5.6	8.2	8.1	78.1	583	3.8
8	+1	−1	+1	5.6	8.2	16.1	70.1	596	5.3
9	+1	+1	−1	5.6	14.1	7.5	72.8	583	5.8
10	+1	+1	+1	5.6	14.1	15.0	65.3	565	6.5
11	0	0	−2	4.1	11.4	3.3	81.2	544	4.5
12	0	0	+2	4.1	11.4	19.1	65.4	574	7.5
13	0	−2	0	4.1	5.0	12.9	78.0	546	5.5
14	0	+2	0	4.1	17.0	11.2	67.7	562	6.3
15	−2	0	0	1.0	11.8	12.4	74.8	631	5.5
16	+2	0	0	7.1	11.1	11.7	70.1	575	6.5
17	0	0	−1	4.1	11.4	7.9	76.6	561	5.5
18	0	0	+1	4.1	11.4	15.7	68.8	607	7.0
19	0	−1	0	4.1	8.3	12.5	75.1	604	4.8
20	0	+1	0	4.1	14.3	11.6	70.0	613	6.5
21	−1	0	0	2.6	11.6	12.2	73.6	629	5.8
22	+1	0	0	5.6	11.2	11.8	71.4	594	5.5

[a] 14% moisture basis.

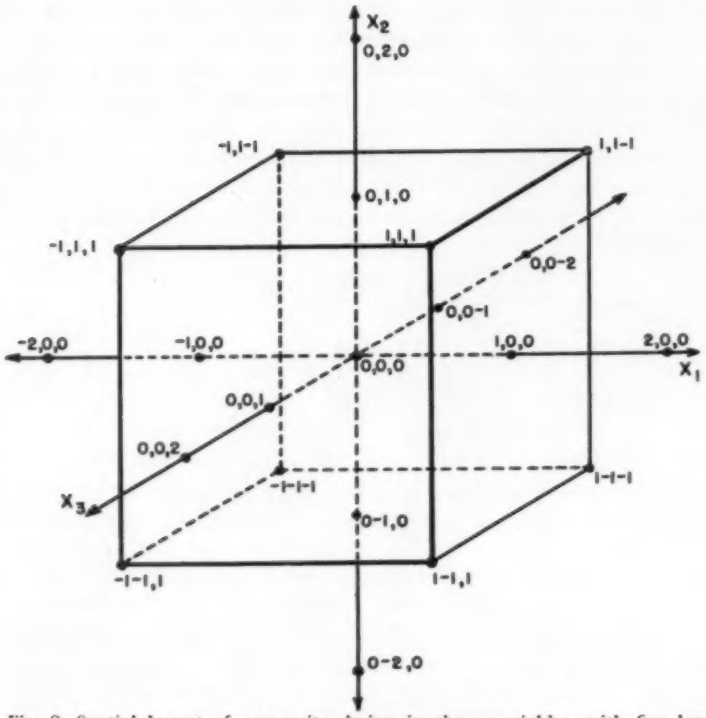

Fig. 2. Spatial layout of composite design in three variables, with five levels. Coded values.

the mean values of two replicates of each treatment, and each replicate was the mean of duplicate cakes prepared from each batter.

Figure 2 illustrates the spatial arrangement of the design. The first two treatments lay at the center, making four replicates altogether of the center point. The next eight treatments formed a three-variable, two-level factorial, the points being located at the vertices of a cube. The next six treatments were placed at a distance of one code unit along the axes, being located at the faces of the cube, and the last six treatments were placed at a distance of two code units along the axes.

Experimental Results

The regression equation obtained from the cake volume data was:

$$Y = 602.487 - 3.556\ X_1 + 3.722\ X_2 + 12.028\ X_3 - 0.709\ X_1^2 - 12.356\ X_2^2 - 12.724\ X_3^2 - 11.063\ X_1\ X_2 - 14.813\ X_1X_3 - 13.438\ X_2X_3,$$

where Y is estimated cake volume, and the values of the independent variables are the coded values of each. Note that in the use of this equation, such as estimating volume for some composition of the reconstituted flour, it is necessary first to compute the ratios from the percentages of fractions, then code the ratios and use the coded values in the equation. The equation may be decoded by inserting code values and collecting terms, but it is more convenient to use it in the form given. The coding simply transfers the origin of coordinates to the center of the design pattern of Fig. 2. We can then note what happens in the volume-concentration relation as departures from the normal are made. The equation may be considered to define a response surface in a four-dimensional space; as flour composition varies continuously, a continuous surface is generated. The regression equation summarizes the experimental volume results and provides the best-fitting second-degree approximation to the true response surface in terms of the experimental values. It may be used to estimate the volumes for compositions not actually included in the study, so long as they are in the ranges actually used and, one might add, not too close to the edges of the ranges. Or, as illustrated below, it may be used to construct model response surfaces as visual aids for examining the volume-concentration changes observed.

Statistical analysis was made by analysis of variance (Table IV). The null hypothesis formulated was that there was no functional variation of cake volume with flour composition. It is evident that the hypothesis must be rejected at the 1% probability level. In the analysis, the error mean square is the random error derived from the

TABLE IV

ANALYSIS OF VARIANCE FOR CAKE VOLUME DATA OF RECONSTITUTED CAKE FLOURS

SOURCE OF VARIANCE	DEGREES OF FREEDOM	MEAN SQUARES
Due to regression	9	
Linear		
X_1	1	455
X_2	1	499
X_3	1	5,208**
Quadratic		
X_1^2	1	23
X_2^2	1	6,982**
X_3^2	1	7,404**
Interaction		
X_1 by X_2	1	1,958**
X_1 by X_3	1	3,511**
X_2 by X_3	1	2,889**
Deviations from regression	11	893**
Error	23	151

replication of treatments. The linear mean squares are measures of the variation accounted for by the linear part of the approximation, and of these only the tailings/starch ratio is highly significant. The quadratic mean squares are the additional variation accounted for by the squared terms in the approximation, and of these only the ratio of water-solubles to the other fractions is not highly significant. The interaction mean squares, all highly significant, are the additional variation accounted for by interaction among flour fractions. The deviation from regression (lack of fit) is a measure of the success of the second-order polynomial to adequately describe the experimental results. The polynomial expansion of the function accounts for 73% of the variation ($R = 0.856$), which is fairly good for a second-order approximation for which no prior evidence was available concerning ranges and scaling of the variables and connected with which there were procedural approximations such as determining optimum liquid levels for each treatment combination. Actually, the random error was satisfactorily small, for cake-baking, with standard deviation $\pm$ 12 cc. The smallness of the experimental error increases the significance of the lack of fit. As mentioned above, a better fit might be obtained by using narrower ranges of the variables or by fitting the data with an equation of third or higher order and taking more experimental points. However, the main contention is demonstrated, that there appears to be variation of cake volume dependent upon the flour composition used.

From the significance of the analysis of variance and the magnitude of the partial regression coefficients, it may be inferred that the response of the X_1 variable is essentially linear, and that as the proportion of water-solubles is increased there is in general a slight decrease in cake volume. The greatest effect of water-solubles lies in conditioning (interaction) the response of the other variables at their extreme values. The responses of the X_2 and X_3 variables are essentially curvilinear; i.e., each tends to have a maximum value. Further, the response of each variable depends upon the levels of the other two. In terms of flour composition this means that there are a number of different compositions which give the same volume, that some compositions yield far larger cakes than others, that the volume obtained depends markedly upon the proportions of the flour fractions, and that some ratios of fractions give volumes very different from others because of interaction among the fractions. In general, the effect of varying composition about the normal percentages is rather small, and very large changes of volume occur only when extreme compositions are used.

Demonstration of these conclusions is a trifle awkward because of the necessity of using ratios which is an unfortunate but apparently necessary limitation. If we differentiate the regression equation with respect to X_1,

$$\frac{\partial Y}{\partial X_1} = -3.556 - 1.418X_1 - 11.063X_2 - 14.813X_3,$$

the expression is an indication of how rapidly the volume is changing with unit change of X_1 at specific levels of X_1, X_2, and X_3. The effect of X_1 depends upon the levels of X_2 and X_3, as indicated by the magnitude of the coefficients of the variables, and quite obviously there is a band of values near the normal composition where the effect of X_1 is very small. with respect to X_2,

$$\frac{\partial Y}{\partial X_2} = 3.722 - 11.063X_1 - 24.712X_2 - 13.438X_3$$

shows how rapidly the surface changes along the X_2 axis for specific values of the three ratios. The change is least at values near the normal composition, and the change of sign as one ranges from low to high values of the ratios indicates that the rate of change of X_2 goes through a minimum near the center values. That is, the volume itself goes through a maximum here. With respect to X_3,

$$\frac{\partial Y}{\partial X_3} = 12.028 - 14.813X_1 - 13.438X_2 - 25.448X_3,$$

and the rate of change of volume with respect to X_3 depends very much on the specific values of the three ratios. As the ratio of tailings to starch increases from the lowest value, the rate of increase of volume, initially very rapid, begins to taper off, although near the normal composition the volume is still rising, and doesn't reach a maximum until the X_3 ratio is larger than normal. Beyond this, as the percentage of tailings increases, the slope falls off. These considerations illustrate that the general volume response surface tends to a maximum and that in general the curvature of the surface is greatest along the axis of the tailings/starch variable. If these three partial derivatives are set equal to zero, and the equations solved simultaneously for the values of the ratios, we find a composition: 6.1% water-solubles, 10.0% gluten, 11.6% tailings, 72.3% prime starch, and a volume of 599 cc. At this point, the slope of the surface is zero, but it is easy to show that there are compositions for which the estimated volume is larger—e.g., 1.0% water-solubles, 12.4% gluten, 18.0% tailings, and 68.6% prime starch. Consequently, the point at which the slope is

TABLE V

CHANGE IN SLOPE OF RESPONSE SURFACE AND CAKE VOLUME ALONG X_3 COORDINATE FOR SETS OF VALUES OF X_1 AND X_2

X_1	X_2	X_3	$\left(\frac{\partial X_3}{\partial Y}\right)_{X_1, X_2}$	Flour Composition [a]				Volume Calculated	Volume Actual	Cake Score
				Water Solubles	Gluten	Tailings	Prime Starch			
				%	%	%	%	cc	cc	
−1	−1	−1	54.7	2.6	8.5	8.3	80.6	525	499	5.3
−1	−1	+1	13.8	2.6	8.5	16.6	72.3	606	595	7.5
0	0	−1	37.5	4.1	11.4	7.9	76.6	578	561	5.5
0	0	+1	−13.4	4.1	11.4	15.7	68.8	602	607	7.0
+1	+1	−1	9.2	5.6	14.1	7.5	72.8	582	583	5.8
+1	+1	+1	−41.7	5.6	14.1	15.0	65.3	550	565	6.5
−1	+1	−1	38.9	2.6	14.5	7.7	75.2	582	566	5.8
−1	+1	+1	−12.0	2.6	14.5	15.5	67.4	608	585	7.0
+1	−1	−1	36.1	5.6	8.2	8.1	78.1	570	583	3.8
+1	−1	+1	−11.8	5.6	8.2	16.1	70.1	591	596	5.3
Comparison:										
									(Mean)	
0	0	0	12.0	4.1	11.4	12.0	72.5	602	592	5.8

[a] 14% moisture basis.

zero is not a true maximum, but a stationary point—in some directions it is a maximum, in others not. It is then the high point of a pass through a ridge, and the general surface is thus a skewed saddle, with the steepest slope downward along the axis of tailings/starch ratio, with a less steep downward slope along the axis of gluten/(tailings + starch) and a mild upward slope along or near to the axis of the ratio of water-solubles to the other fractions.

This rough analysis indicated also that the mode of change of cake volume depended very much upon the actual composition and that some combinations of fractions produced significantly greater degrees of change than did others; i.e., interaction was involved. The relative effect of interaction can be assessed from the magnitude of the partial regression coefficients; the relative effect of each ratio can be similarly assessed, and the compositions at which interaction becomes great can be determined by trying values in the regression equation. It is apparent that the tailings-to-starch ratio had the greatest effect on cake volume, that the ratio of gluten to tailings and starch had considerable effect, that the water-solubles ratio had little effect, and that interaction became greater as compositions deviated from near the normal one.

We are most interested in what the response is like near the center of the design, in the coded region $-1 \leq X_i \leq +1$. It would be expected, perhaps, that peculiar compositions at the extremes of the composition ranges would be associated with cakes of substandard volume. As an illustration, if we investigate changes in the X_3 ratio,

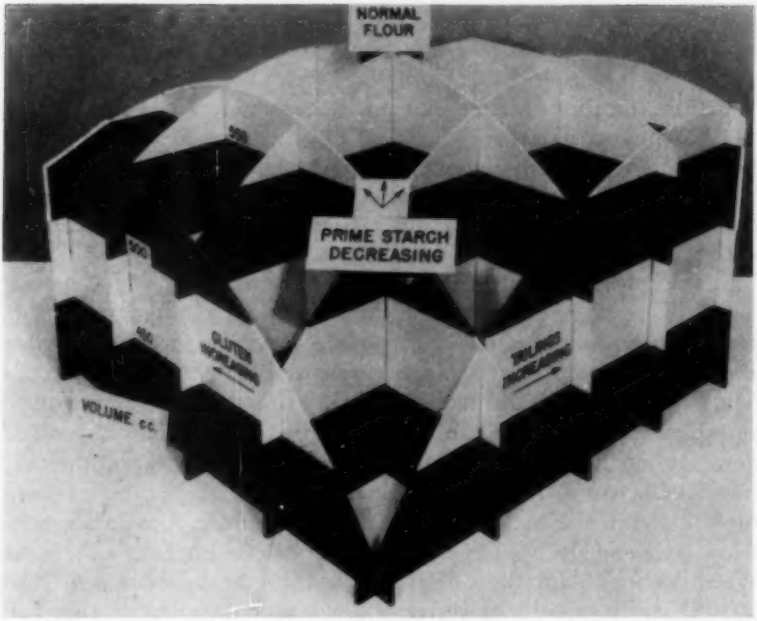

Fig. 3. Three-dimensional model showing changes in cake volume with changes in variables X_2 (indicated as gluten increasing) and X_3 (indicated as tailings increasing). Variable X_1 is constant at the median value of its range. Variables X_2 and X_3 extend from −2 to +2 (coded values).

for successive sets of fixed values of X_1 and X_2, this will show the effect of tailings and starch on cake volume for particular flour compositions. Table V is a tabulation of volume changes, cake scores, and values of the slopes of the surface with respect to the tailings-to-starch ratio for a number of treatment combinations in the range in question. These are ranges of composition well among those typical of soft wheat flours. It is noticeable, though not always the case, that relatively large volumes and high scores (good internal structure) are associated with an increased percentage of tailings. The magnitude and sign of the slope in the X_3 direction for each treatment indicates how rapidly the surface is changing in the tailings direction at that composition.

As mentioned above, the regression equation can be interpreted as defining a volume response surface in a four-dimensional space. Figure 3 illustrates a model constructed as a sample surface, to show the effect of varying two ratios at a time (in order to permit three-dimensional representation) and to show the accompanying effects. In this

case, the ratio of water-solubles to the other fractions was set at the normal value of its range, since the water-solubles ratio was the one having the least effect. The tailings-to-starch ratio is plotted as "Tailings Increasing" against the gluten-to-tailings-and-starch ratio plotted as "Gluten Increasing." The vertical direction is cake volume in cc. It will be noted that as gluten or tailings or both increase, the amount of prime starch decreases. In most of the planes taken at right angles to the axes across the surface, the volume goes through a maximum, and in fact the surface itself has a maximum. The point of largest volume on this particular surface, 606 cc., occurs where the composition is: 4.1% water-solubles, 11.0% gluten, 14.2% tailings, and 70.7% prime starch. Note that the percentage of tailings is somewhat greater and the percentages of gluten and starch are slightly less than normal. The model illustrates the necessity of specifying the values of the other variables when we wish to discuss the effect of a particular component. For example, if the gluten is maintained at a high level, and the tailings are increased, the volume increases only slightly, then declines rapidly (down the back of the figure), but if gluten is maintained at a low level and the tailings are increased, volume increases steadily to a maximum. Tables VI and VII incorporate the association of cake volume and compositions corresponding to points along the median planes to this surface.

Table VI shows cake volumes obtained with five combinations of fractions taken with the ratio of gluten to total starch variable. The other two ratios are constant. The composition of the middle column is that of the "normal" flour. The amount of gluten varies from a smaller than normal to a larger than normal amount. Although the ratio of tailings to starch is constant, the actual amounts present change somewhat as the gluten changes. As percent gluten increases,

TABLE VI

ACTUAL AND ESTIMATED CAKE VOLUMES FOR SEVERAL TREATMENT COMBINATIONS, WITH GLUTEN-TO-TOTAL-STARCH RATIO VARIABLE

Cake volume, cc.					
Actual	546	604	596	613	562
Estimated	546	586	602	594	560
Flour protein, %[a]	4.7	6.7	8.5	10.3	11.9
Treatment No.	13	19	1	20	14
Composition, %[a]					
Water-solubles	4.1	4.1	4.1	4.1	4.1
Gluten	5.0	8.3	11.4	14.3	17.0
Tailings	12.9	12.5	12.0	11.6	11.2
Prime starch	78.0	75.1	72.5	70.0	67.7

[a] 14% moisture basis.

TABLE VII

ACTUAL AND ESTIMATED CAKE VOLUMES FOR SEVERAL TREATMENT COMBINATIONS, WITH TAILINGS-TO-PRIME-STARCH RATIO VARIABLE

Cake volume, cc.					
Actual	544	561	596	607	574
Estimated	528	578	602	602	576
Flour protein, %[a]	8.3	8.4	8.5	8.7	8.8
Treatment No.	11	17	1	18	12
Composition, %[a]					
Water-solubles	4.1	4.1	4.1	4.1	4.1
Gluten	11.4	11.4	11.4	11.4	11.4
Tailings	3.3	7.9	12.0	15.7	19.1
Prime starch	81.2	76.6	72.5	68.8	65.4

[a] 14% moisture basis.

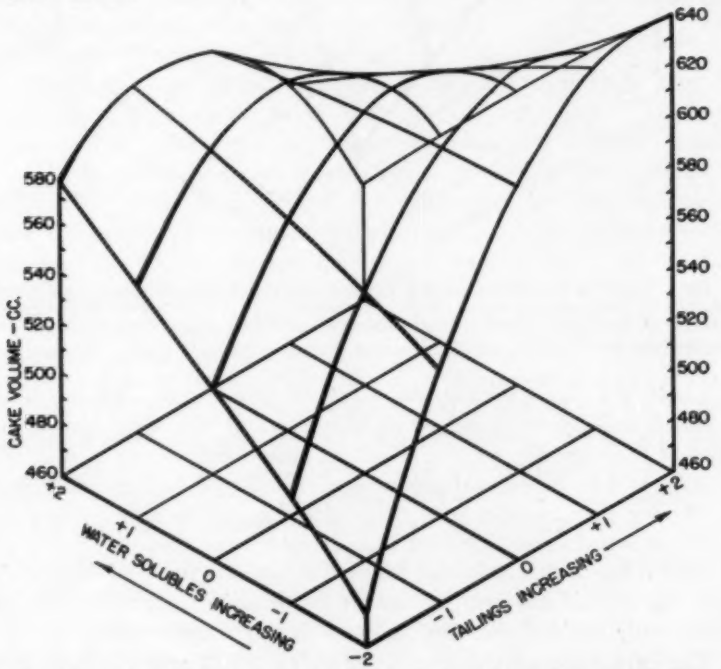

Fig. 4. Drawing of response surface showing cake volume changes with changes in variables X_1 (indicated as water-solubles increasing) and X_3 (indicated as tailings increasing). Variable X_2 is fixed at the median value of its range. Coded values of the variables are shown.

the cake volume increases to a maximum of 602 cc. predicted value, then decreases. Since the protein content increases with increasing concentration of gluten, there would *appear* to be no simple association between protein level and cake volume.

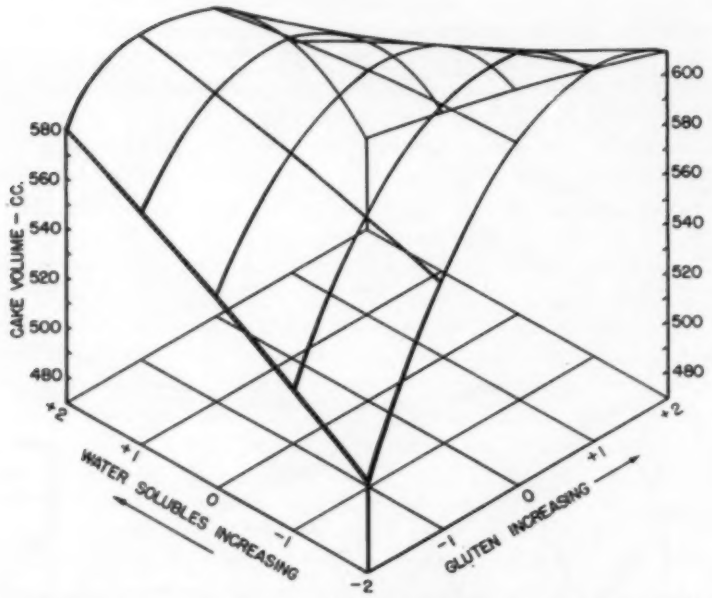

Fig. 5. Drawing of response surface showing cake volume changes with changes in variables X_1 (indicated as water solubles increasing) and X_2 (indicated as gluten increasing). Variable X_3 is fixed at the median value of its range. Coded values of the variables are shown.

Table VII shows volumes of the set of cakes baked with the ratio of starch tailings to prime starch variable and the other two ratios fixed at their median values. That is, water-solubles and gluten are constant and in the normal percentages. The starch tailings and prime starch vary over a rather wide range. Here again the cake volume increases to a maximum, then falls off sharply. Note that the largest estimated value of this series has somewhat more tailings and less starch than the normal amounts. Although the protein levels of the flours change only slightly, the cake volumes change considerably.

The experimental results recorded in Tables VI and VII show that there did not seem to be much association between cake volume and the level of any one component. There must have been complex interaction among the components that accounted for volume changes.

Figure 4 illustrates another example of a three-dimensional surface; this is a saddle type of surface generated if the gluten-to-total-starch ratio is held constant and the ratios of tailings to starch and of water-solubles to gluten, tailings, and starch are varied. Changes in the

amount of water-solubles with tailings/starch at a fixed value lead to an essentially linear volume change, although with the tailings/starch near the normal value (along the ridge of the surface) there is a negligible change in volume. However, if the water-solubles ratio is fixed, the change in volume due to changing tailings and starch is markedly curvilinear.

A similar saddle surface is shown in Fig. 5. In this plot the tailings-to-starch ratio is held constant. The water-solubles and gluten ratios are varied. It may be noted that here, also, the effect of water-solubles is essentially linear, and with nearly normal amounts of gluten, the effect on volume is small. However, at the extremes of the amount of gluten, change in the quantity of water-solubles produces rather large changes in volume. Change due to the variation of the gluten ratio is markedly curvilinear.

Figure 6 was drawn to show typical effects of altering the proportions of two fractions at a time. In Fig. 6, A, where tailings and starch were fixed at the normal values and water-solubles and gluten were varied, the small decrease in volume as water-solubles increased is shown. For *these* compositions there was relatively little change in cake volume, except at the very high gluten, very low water-solubles composition. In Fig. 6, B, where starch and gluten concentrations were fixed and water-solubles and tailings were varied, there was a steady decrease in volume as tailings decreased, or water-solubles increased. In Fig. 6, C, tailings and water-solubles were fixed and starch and gluten were varied. There was a large volume change. Volume was low at both high gluten-low starch and at high starch-low gluten, and the greatest volume was found near the normal amounts of both. In Fig. 6, D, water-solubles and starch were fixed at the normal amounts and gluten and tailings were varied. Here also, volume was greatest near the normal amounts and fell at extreme compositions.

It was apparently not possible to obtain quantitative measures of the magnitude of response of each component, because of the presence of significant interactions. But it was possible to generalize concerning the direction and relative magnitude of response of the components. Water-solubles tended to decrease layer volume, and the extent of decrease, although small, was approximately linear with the concentrations used. Variation of gluten and prime starch concentration had a rather complex and nonlinear effect on cake volume. A small increase in gluten or a small decrease in prime starch concentration increased layer volume slightly, but substantially greater or smaller amounts of either resulted in much smaller cakes. Tailings acted to increase vol-

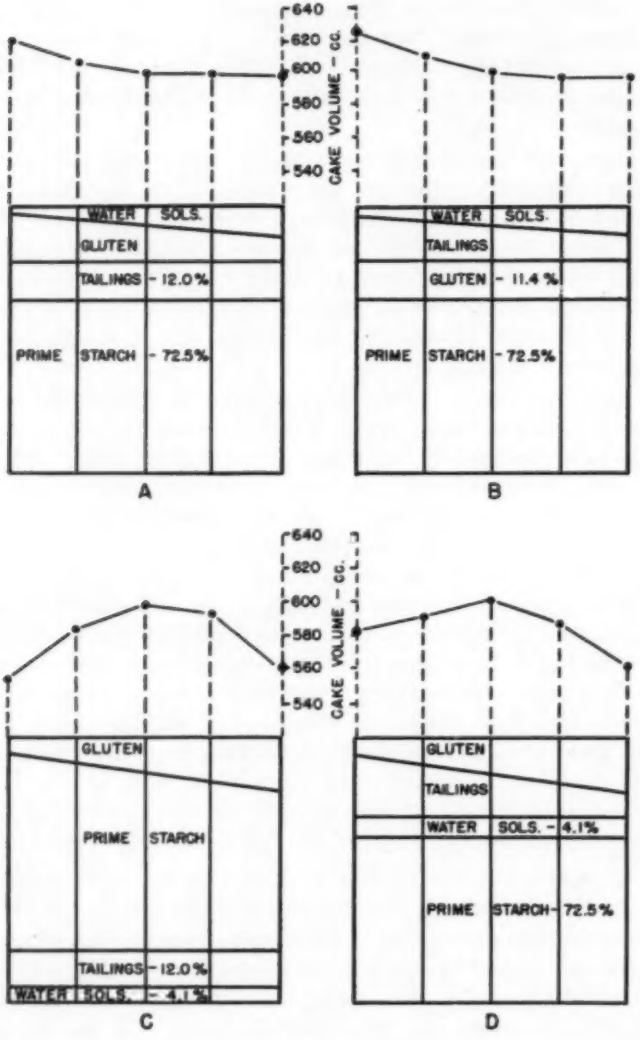

Fig. 6. Graphs of calculated cake volumes for various flour compositions. Relative amounts of flour components are indicated by lengths of vertical intercepts on the bar graph below each graph. A, water-solubles vary from 1.0 to 7.2%; gluten from 14.5 to 8.3%. B, water-solubles vary from 1.0 to 7.2%; tailings from 15.1 to 8.9%. C: gluten varies from 6.0 to 16.8%; prime starch from 77.9 to 67.1%. D: gluten varies from 6.0 to 16.8%; tailings from 17.4 to 6.6%.

ume and to produce a finer internal structure, and generally the larger cakes contained large amounts of tailings. But for any specific change in concentration, it is not possible to point out the effect of altering the quantity of one component without concomitantly taking into account the levels and changes of levels of the other three, since the response depends upon the concentrations of all the fractions. For example, if one were to increase the tailings concentration considerably at the expense of the prime starch, the resultant cake might be rather different from that obtained if one increased the tailings concentration by spreading the adjustment in ratios over all the other fractions. In particular it was shown that, for the materials utilized, reconstituted flours with compositions not very different from the normal composition could yield cakes with volumes significantly different from layers baked from the normal flour. These results bring into question laboratory analytical techniques such as enriching a flour with an added component, since the result of such treatment may be occasioned by the effect of the change in composition as well as by the "inherent" effect of the added component.

Such differences as were found from one treatment to another cannot be ascribed to differences in quality in its usual sense, since all treatment combinations were drawn from a common source with respect to inherent chemical makeup. Nor can such differences be attributed to protein content, for the results of the experiment indicate that protein *per se* had little association with the responses of treatment combinations used. Some of the better cakes contained 10.0 to 10.5% protein, yet cakes of equal volumes were very low in protein. (Compare Tables VI and VII.) Apparently the balance of the fractions was the main factor determining volume, and total protein content depended merely upon the protein distribution of the fractions. Similarly, gluten protein content cannot be considered, for these materials, as the major cause for volume differences, since all the series of cakes presented in Table VII contained the same amount of gluten. Evidently, given a particular set of flour fractions, the proportioning of these fractions was the limiting factor involved in the production of a superior cake, at least when good-quality flour components were used. Consequently, one may infer that one aspect of some importance in the analysis of cake flour quality is the balance of flour components in the flour.

Acknowledgments

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Literature Cited

1. Adams, G. A. Separation of starch and gluten. VI. Starch recovery and purification. Can. J. Technol. **29:** 217–231 (1951).
2. Box, G. E. P. The exploration and exploitation of response surfaces: Some general considerations and examples. Biometrics **10:** 16–60 (1954).
3. Box, G. E. P., and Youle, P. V. The exploration and exploitation of response surfaces: An example of the link between the fitted surface and the basic mechanism of the system. Biometrics **11:** 287–323 (1955).
4. Box, G. E. P., and Hunter, J. S. Experimental designs for the exploration and exploitation of response surfaces. *In* Experimental designs in industry, a symposium, Nov. 5–9, 1956, ed. by V. Chew; pp. 138–190. Wiley: New York (1958).
5. Cochran, W. G., and Cox, Gertrude M. Experimental designs (2nd ed.), chap. 8A. Wiley: New York (1957).
6. Davies, O. L., ed. The determination of optimum conditions. *In* The design and analysis of industrial experiments (2nd ed., rev.); chap. XI. Hafner Pub. Co.: New York (1956).
7. Hackler, W. C., Kriegel, W. W., and Hader, R. J. Effect of raw-material ratios on absorption of whiteware compositions. J. Am. Ceramic Soc. **39:** 20–25 (1956).
8. Hader, R. J., Harward, M. E., Mason, D. D., and Moore, D. P. An investigation of some of the relationships between copper, iron, and molybdenum in the growth and nutrition of lettuce. I. Experimental design and statistical methods for characterizing the response surface. Proc. Soil Sci. Soc. Am. **21:** 59–64 (1957).
9. Kissell, L. T. A lean-formula cake method for varietal evaluation and research. Cereal Chem. **36:** 168–175 (1959).
10. Sollars, W. F. Fractionation and reconstitution procedures for cake flours. Cereal Chem. **35:** 85–99 (1958).
11. Sollars, W. F. Cake and cookie flour fractions affected by chlorine bleaching. Cereal Chem. **35:** 100–110 (1958).
12. Yamazaki, W. T. The concentration of a factor in soft wheat flours affecting cookie quality. Cereal Chem. **32:** 26–37 (1955).
13. Zaehringer, Mary V., Briant, Alice M., and Personius, Catherine J. Effects on baking powder biscuits of four flour components used in two proportions. Cereal Chem. **33:** 170–180 (1956).

STUDIES ON THE BEHAVIOR OF ACTIVE DRY YEAST IN BREADMAKING[1]

J. G. Ponte, Jr.,[2] R. L. Glass, and W. F. Geddes

ABSTRACT

A series of comparative studies was made of active dry yeast (ADY) and compressed yeast which had been suspended, prior to use in doughs, at temperatures from 5° to 50°C. When ADY was rehydrated at 40°C. a minimum (5% of the dry weight) of material was leached from the cells. At this temperature of rehydration, baking performance was optimum and comparable to that of compressed yeast. ADY doughs required a shorter mixing time and higher levels of oxidizing improver than compressed yeast doughs. As the temperature of rehydration of ADY was lowered from 40°C. the amount of material leached from the cell increased to a maximum of 29% of the dry weight of the cells at 5°C. Nitrogenous compounds leached from ADY increased from 3.9 to 11.7 mg. nitrogen per g. yeast and glutathione from 2.2 to 9.9 mg. per g. yeast. No glutathione was found in the compressed yeast supernatants, and nitrogen appeared only in relatively small quantities at all temperatures tested. Two distinct effects resulted as the temperature of rehydration of ADY was lowered. The doughs became progressively more sticky and difficult to handle. Rheological tests showed a marked increase in extensibility and a lessened response to oxidizing agents. Identical results could be obtained by the addition of glutathione to doughs prepared using optimally rehydrated ADY. Gas production by the ADY was progressively decreased as the temperature of rehydration was lowered. This resulted in greatly decreased loaf volumes in the baked bread. This was undoubtedly due to inactivation of the cells as they progressively became more disorganized owing to the loss of cellular constituents. Compressed yeast was quite indifferent to the temperature at which it was suspended, prior to use, at all temperatures up to 40°C. At 50°C. considerably decreased loaf volumes resulted from thermal inactivation of the cells, as was also the case with ADY.

Although active dry yeast (ADY) has been in use by bakers since World War II, there is very little information in the literature concerning its baking properties. Much of the research reported on ADY has concerned its rehydration. Jorgensen (8) observed that significant amounts of glutathione appeared in the supernatant when dried yeast was suspended in water. Peppler and Rudert (10) demonstrated that this was a function of the water temperature and that if the temperature was lowered to 5°C. about one-third of the yeast cells were inactivated and a marked decrease in baking performance re-

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sulted. Herrera *et al.* (5) observed that the suspension of ADY in water at 4.5°C. resulted in the loss of large amounts of yeast solids which included virtually all of the soluble nitrogenous compounds of the cell. Sant and Peterson (11) attempted, without success, to minimize these losses by reconstituting the cells in a concentrated extract of dried yeast. However, if the ADY was first rehumidified to a moisture level of 25% by a process of "vapor rehumidification," the effect of subsequent low-temperature rehydration on nitrogen loss by the cells was eliminated.

Herrera *et al.* (5) postulated that the cell wall of ADY becomes badly disorganized by drying and must be completely rehydrated to resume its function as a semipermeable membrane. The hydration process is probably slow at low temperatures, and a loss of cellular material might be expected before the cell wall begins to function properly.

This paper reports the results of an investigation of some of the properties of ADY in comparison with compressed yeast. Rehydration temperature as it affects the leaching of material from the cells was studied. The subsequent effects of the leached material on dough properties, as well as the loss of this material on the ultimate behavior of the cells, were investigated using baking tests, gassing power studies, and instrumental evaluations of dough rheological properties.

Materials and Methods

Yeast. Active dry yeast (ADY) and fresh compressed yeast manufactured by the Red Star Yeast and Products Co., Milwaukee, were used.[3] The ADY, containing 7.09% nitrogen (dry basis), was vacuum-packed in metal cans which were held at −10°C. prior to use. After a can had been opened the unused yeast was kept at −10°C. in tightly stoppered flasks until needed. Two weeks after a can was opened, any remaining yeast was discarded. The compressed yeast, containing approximately 9.0% nitrogen (dry basis), was obtained commercially and delivered fresh weekly. It was kept under refrigeration and any remaining yeast was discarded at the end of the week.

The active dry yeast (ADY) (5 g. containing 8.42% moisture), or compressed yeast (15 g. containing 69.43% moisture), was suspended in water (100 ml.) at temperatures ranging from 5° to 50°C. for 20 minutes. The suspensions were centrifuged for 15 minutes in an International Centrifuge (size 2) at 3000 r.p.m. and the supernatant liquid decanted and assayed for total solids, ash, nitrogen, and glutathione.

[3] The yeasts were provided by the Red Star Yeast and Products Company as representative of their commercial production.

TABLE I

FORMULAS FOR THE STRAIGHT DOUGH AND SPONGE AND DOUGH BAKING PROCEDURES

INGREDIENTS	STRAIGHT DOUGH	SPONGE AND DOUGH		
		Sponge	Dough	Total
	g	g	Dg	g
Flour (14% moisture basis)	200.0	130.0	70.0	200.0
Water	as required	as required		
ADY[a]	3.0	1.8	.....	1.8
Compressed yeast[a]	6.0	4.5	.....	4.5
Potassium bromate	0.002	.....	.....	.....
Dough improver[b]				
ADY doughs	.....	1.0		1.0
Compressed yeast doughs		0.75		0.75
Sodium chloride	2.0	.....	4.0	4.0
Sucrose	10.0	.....	8.0	8.0
Nonfat dry milk[c]			8.0	8.0
Shortening[d]			6.0	6.0

[a] Either one or the other yeast was used in individual tests.
[b] A commercial product which contains 0.27% of oxidizing improver (potassium bromate).
[c] A spray-dried product specially prepared for use in baking.
[d] A hydrogenated vegetable shortening containing emulsifying agents.

Flour. A Southwest Bakers' Patent flour (11.3% protein and 0.45% ash, both on a 14% moisture basis) was used in all of the baking, gassing power, and farinograph studies. A Southwest Bakers' Patent flour (10.5% protein and 0.4% ash on a 14% moisture basis) was used for the extensigraph studies.

Total Solids. The AOAC method for determining total solids of milk (2) was employed in estimating the amount of solids leached from the yeasts at various temperatures. This involved pipetting a suitable aliquot of the centrifuged supernatants into tared aluminum moisture dishes, and heating for 3 hours in a forced-draft air oven at 100°C. Results are expressed as mg. total solids per g. yeast (dry weight basis).

Ash. The dried yeast extracts were analyzed for ash by the magnesium acetate method described in *Cereal Laboratory Methods* (1).

Glutathione. The method of Woodward and Frey (12) was used for the determination of glutathione in extracts of ADY and compressed yeast. This titrimetric method involves the oxidation of sulfhydryl groups with potassium iodate using a starch indicator.

Nitrogen. The Kjeldahl procedure (1) was used to determine nitrogen in yeast extracts.

Baking Tests. The formulas for the straight dough and sponge and dough procedures are shown in Table I.

For the straight doughs the ingredients were mixed for 3.5 minutes (unless otherwise indicated) in a Swanson mixer equipped with a McDuffee bowl. Absorption was 66% on a 14% moisture basis. The

dough (scaled to 150 g.) was fermented at 30°C. for 95 minutes, punched by passing through the National Dough Sheeter (rolls set at 9/32 in.), rounded, and fermented for an additional 25 minutes. The dough was then passed twice through the sheeting rolls with settings at 9/32 and 5/32 in. respectively. It was then molded on wooden molding rolls, proofed for 55 minutes at 30°C. and baked in a rotary hearth oven for 25 minutes at 227°C. Loaf volumes were measured 1 hour after baking by rapeseed displacement, and the bread was scored the following day.

For the sponge and dough procedure the sponges were mixed at 26°C. in a Swanson mixer with a McDuffee bowl, and fermented for 4 hours at 30°C. The sponges were then returned to the mixer, the remaining ingredients added and mixed from 1.5 to 5.0 minutes using an absorption of 63% (flour basis at 14% moisture). The doughs were fermented 20 minutes at 30°C., punched with the dough sheeter as for the straight doughs, and refermented for 15 minutes at 30°C. The doughs were molded as described for the straight doughs, pan-proofed to a height of 2.4 cm. above the bread pan, and were baked, measured, and scored as described for straight doughs.

Farinograph Studies. Three-hundred grams of flour (14% moisture basis), adjuncts as described later, and distilled water were mixed in the farinograph for 20 minutes at 30°C. Sufficient water was used to center the curve on the 500 Brabender unit (B.u.) line at maximum dough development. Two measurements were made of the farinograms; namely, dough development time, the time in minutes from the start of mixing required to reach a maximum consistency of 500 B.u., and the "drop-off" value, a measurement related to the Tolerance Value, the difference in Brabender units from the 500 B.u. line to the center of the curve 20 minutes after the addition of water.

Gas Production. Gas production curves were made at 30°C. for 6 hours on doughs (150-g.) prepared by the sponge and dough and straight dough procedures, already described, using the Chopin Zymotachygraphe (9). The results are means of duplicate determinations corrected to S.T.P.

Extensigraph Studies. The structural relaxation method described by Hlynka (6) was used to study the effects of various adjuncts including extracts of ADY rehydrated at various temperatures, on the physical properties of the doughs.

Doughs were made with 200 g. flour (14% moisture basis), 1% sodium chloride (flour basis), 55% total absorption (flour basis), and with additives in solution as indicated. The dough additives consisted of yeast extracts prepared at various temperatures as already de-

scribed, potassium bromate, and glutathione (GSH).

Prior to mixing, the flour was flushed with nitrogen and kept overnight under nitrogen to remove adsorbed oxygen. The doughs were then mixed for 3 minutes under an atmosphere of nitrogen in a specially constructed mixer adapted from a design developed at the Grain Research Laboratory (7), and brought from the mixer at a temperature of 30°C.

After mixing, the doughs were divided into two 150-g. pieces and placed in a humidified (85% relative humidity) fermentation cabinet at 30°C. for periods ranging from 5 minutes to 4 hours (reaction time). They were then shaped on the extensigraph, clamped into cylinders, and returned to the fermentation cabinet for periods ranging from 5 minutes to 3 hours (rest period). Following the rest period, the dough pieces were stretched on the extensigraph and a curve obtained.

Results

Leaching Studies. The effects of suspending ADY and compressed yeast in water at various temperatures on the loss of various constituents from the cells are recorded in Table II. The temperature of rehydration markedly influenced the loss of cell material from ADY, whereas compressed yeast was relatively insensitive to the temperature of the suspension.

The amount of nitrogen leached from ADY increased threefold as the rehydration temperature was lowered from 40° to 5°C., while the total solids lost increased almost sixfold over the same temperature range. A possible explanation for this is that at 40°C. the yeast

TABLE II

EFFECT OF TEMPERATURE OF PREPARATION ON LOSS OF MATERIAL FROM COMPRESSED AND ACTIVE DRY YEAST

Temperature of Rehydration [a]	Material Leached from Cells							
	Solids [b]		Ash [b]		Nitrogen [b,c]		Glutathione [b]	
	ADY	Comp.	ADY	Comp.	ADY	Comp.	ADY	Comp.
°C.								
5	288	8.7	29.5	2.2	11.7	0.7	9.9	0.0
10	258	8.9	26.2	1.4	10.4	0.6	8.0	0.0
20	144	8.3	13.8	1.8	7.5	0.9	5.7	0.0
30	56	10.1	4.2	2.4	4.3	0.7	2.2	0.0
40	51	14.2	3.9	2.7	3.9	0.6	2.2	0.0
50	79	21.9	6.2	6.1	6.2	1.2	3.4	0.0

[a] The cells were in contact with water for 20 minutes prior to centrifugation. Longer extraction was without effect.
[b] All values are mg. per g. yeast (dry basis).
[c] Nitrogen content of intact cells (dry wt. basis). ADY 71 mg. per g., compressed yeast 64.5 mg. per g.

TABLE III

EFFECT OF MIXING TIME ON LOAF VOLUMES[a] OF BREAD MADE FROM STRAIGHT DOUGHS CONTAINING ACTIVE DRY YEAST OR COMPRESSED YEAST

Mixing Time	Loaf Volume[b]	
	Doughs Made with 3% Compressed Yeast[c,d]	Doughs Made with 1.5% ADY[c,d]
minutes	*cc*	*cc*
1.5	604	647
2.0	635	671
2.5	681	701
3.0	717	726
3.5	752	736
4.0	772	712
4.5	762	718
5.0	768	733

[a] Results are means for duplicate doughs of two loaves each.
[b] Least significant difference, 25 cc.
[c] Flour basis.
[d] ADY was rehydrated by suspension in water for 20 minutes at 40°C. Compressed yeast was prepared at 30°C.

cells selectively retain some non-nitrogenous compound such as trehalose, a selectivity which is lost as the rehydration temperature is lowered. Glutathione and ash remained a rather constant percentage of the total material leached over the temperature range studied. Glutathione was not present in measurable amounts in the supernatants of the compressed yeast suspensions.

Baking Studies. Straight dough procedure: Preliminary baking studies indicated that a level of ADY of 1.25 to 1.50% (flour basis) produced a loaf of bread comparable to that made with 3% compressed yeast when a rehydration temperature of 40°C. was used. The doughs made with ADY developed more rapidly than those made with compressed yeast, particularly at the higher levels of ADY. In general, the optimum doughs made with ADY were smoother and more extensible than the doughs made with compressed yeast and produced loaves with a more even break and shred. The loaves with compressed yeast, however, scored higher in crumb grain and texture.

The loaf volumes for different mixing times in Table III show that doughs made with ADY require a shorter mixing time to give optimal loaves than doughs made with compressed yeast. All the doughs, but particularly those made with ADY, became more pliable and extensible as mixing time was increased. However, while the ADY doughs produced a loaf of maximum volume with 3.5 minutes of mixing and began to appear overmixed after that time, the compressed yeast doughs produced bread of a significantly greater volume

TABLE IV

INFLUENCE OF TEMPERATURE OF SUSPENSION AND OF GLUTATHIONE UPON LOAF VOLUMES OF BREAD[a] MADE FROM DOUGHS USING YEAST CELLS AND THE SUPERNATANT OF ACTIVE DRY YEAST SUSPENSION[b]

Suspension Temperature	(Straight Dough Process) Loaf Volumes[c,d]					
	A	B	C	D	E	F
°C.	cc	cc	cc	cc	cc	cc
5	484	505	715	742	631	705
10	573	653	726	746	675	728
20	684	728	752	747	682	721
30	728	729	759	752	724	746
40	741	743	762	751	724	746
50	711	723	755	713	703	732

[a] The results are means of duplicate bakes of two loaves each.
[b] 1.5% and 3.0% ADY and compressed yeast (flour basis) respectively.
[c] Required for significant difference (5% level), 25 cc.
[d] Doughs made with:
A, entire ADY suspension;
B, ADY cells only, rehydration liquid removed;
C, 3% compressed yeast to which were added supernatants from B;
D, entire compressed yeast suspension;
E, ADY rehydrated at 40°C., to which had been added glutathione to bring the level to that of series A;
F, compressed yeast rehydrated at 40°C., to which had been added glutathione to bring the level up to that of series A.

upon longer mixing and appeared to be much more tolerant to overmixing.

The effect of low-temperature rehydration of ADY upon baking behavior was quite marked (Table IV, series A). At rehydration temperatures below 30°C. a highly significant decrease in loaf volume occurred; in addition, these doughs were extremely sticky and difficult to handle. In contrast, the loaf volumes with compressed yeast (series D) were not influenced by the temperature of suspension below 50°C.; at this temperature, however, there was a significant decrease in loaf volume, owing, probably, to thermal killing of the cells.

Removal of the ADY cells from the rehydration liquid and their incorporation into doughs (series B) resulted in the disappearance of the stickiness and a significant increase in loaf volume of the bread baked from ADY suspended at 10° and 20°C. The addition of the suspension liquids obtained from this series to doughs made with compressed yeast (series C) produced a significant decrease in loaf volume only when the supernatant was prepared at 5°C. The stickiness of doughs, observed with ADY rehydrated at 5°, 10°, and 20°C. in series A but which was absent in series B, was again in evidence in series C.

That at least part of the loaf volume depressant effect of low-temperature rehydration was due to glutathione (GSH) present in the supernatant is shown by series E and F. These were breads

baked with optimally prepared ADY and compressed yeast, respectively, to which GSH had been added in amounts found in ADY supernatants rehydrated at the indicated temperatures. At temperature equivalents below 30°C., ADY doughs produced loaves whose volumes were significantly depressed. In addition, the doughs were very sticky and difficult to handle. Compressed yeast doughs were much less sensitive to the presence of GSH and it was only at a temperature equivalent of 5°C. that a significant decrease in volume was obtained. As with ADY, however, very sticky doughs were obtained when GSH was present with compressed yeast at temperature equivalents below 30°C.

The loaf volume response of straight doughs baked with ADY or compressed yeast to various levels of potassium bromate and potassium iodate was studied. In both cases (Fig. 1) a higher level of the oxidizing improver was required to obtain the maximum response in bread baked with ADY. With compressed yeast, for example, the maximum loaf volume was obtained using 10 p.p.m. potassium bromate, after which the volumes began to decrease; whereas with ADY the maximum loaf volume was obtained with 30 p.p.m. potassium bromate. There was no significant difference in loaf volumes of bread made with ADY and compressed yeast when optimal bromate levels were used with each yeast.

Similarly, with potassium iodate more of the oxidizing agent was required to produce optimum loaf volume when ADY was employed as the leavening agent; the level of iodate which produced the opti-

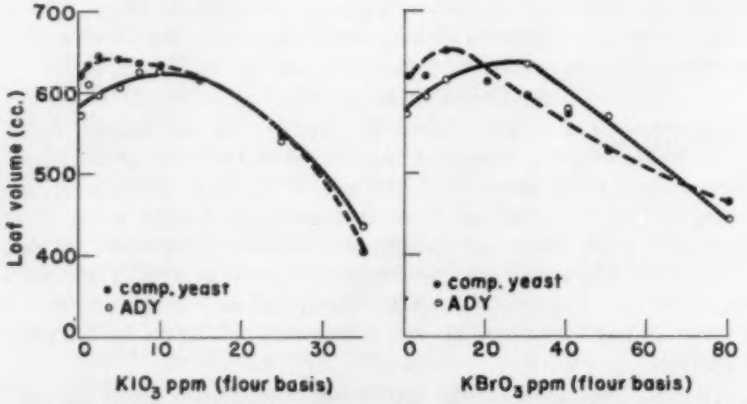

Fig. 1. Effect of potassium bromate and potassium iodate on the loaf volume of bread prepared using compressed yeast and active dry yeast (ADY) by the straight dough procedure. The ADY was rehydrated at 40°C. for 20 minutes.

mum loaf of bread with ADY produced overoxidized doughs when used with compressed yeast.

These results cannot be entirely explained on the basis of glutathione leached from ADY, even when rehydration is carried out at optimum temperatures. To produce optimum loaf volume with ADY an additional 7×10^{-4} meq. potassium bromate per g. flour were required. However, calculations based on the data of Table II show that only about 1×10^{-4} meq. of glutathione per g. flour would appear as a result of the rehydration of the ADY. Again there was no significant difference between the maximum loaf volumes obtained with the two types of yeast.

Sponge and Dough Procedure. The results of baking tests carried out by the sponge and dough method in which dough mixing time was varied are summarized in Table V.

The doughs containing compressed yeast gave bread of somewhat larger volume, and required slightly less time to pan-proof to the same height as the doughs made with ADY.

The doughs employing ADY "picked up" on the mixing hooks after about 2 minutes of mixing time, while those made with compressed yeast required approximately 0.5 minute longer mixing time to reach this stage of dough formation. The doughs made with ADY appeared to be smoother in appearance than those made with compressed yeast. All the bread was of good quality. Better bread was produced over a wider range of mixing time using the sponge and dough method than was obtained by the straight dough procedure.

TABLE V

EFFECT OF VARIATION IN MIXING TIME ON BREAD[a,b] MADE WITH ACTIVE DRY YEAST AND COMPRESSED YEAST USING THE SPONGE AND DOUGH PROCESS

Dough Mixing Time	Loaves Made with ADY		Loaves Made with Compressed Yeast	
	Proof[c] Time	Loaf Volume[d]	Proof Time[c]	Loaf Volume[d]
minutes	*minutes*	*cc*	*minutes*	*cc*
1.5	95	795	92	803
2.0	87	828	81	830
2.5	86	855	82	893
3.0	92	850	82	880
3.5	89	838	83	885
4.0	91	858	78	873
4.5	102	895	82	883
5.0	97	863	77	860

[a] Results with single doughs consisting of two loaves.
[b] Respectively, 0.9% ADY (rehydrated at 40°C.) and 2.25% compressed yeast (suspended at 30°C.), flour basis.
[c] Dough proofed to a constant height of 2.4 cm. above pan.
[d] Least significant difference 25 cc.

TABLE VI

INFLUENCE OF TEMPERATURE OF PREPARATION ON GAS PRODUCTION BY ADY AND COMPRESSED YEAST IN STRAIGHT DOUGHS[a]

Yeast Suspension Temperature	Gas Production for 6 hours at 30°C.[b]					
	A	B	C	D	E	F
°C.	cc	cc	cc	cc	cc	cc
5	1,300	1,225	1,985	1,920	1,870	2,040
10	1,455	1,395	1,910	1,930	1,845	2,020
20	1,845	1,805	1,935	1,905	1,930	1,930
30	1,975	1,915	1,925	1,970	1,970	1,945
40	1,985	1,915	1,950	1,920	1,895	1,920
50	1,860	1,840	1,965	1,615	1,620	1,930

[a] 1.5% ADY or 3.0% compressed yeast (flour basis).
[b] Means of duplicate determinations. Least significant difference, 32 cc.
[c] Doughs made with:
A, entire ADY suspension;
B, ADY cells only.
C, ADY rehydrated at 40°C., and with the supernatants from group E.
D, entire compressed yeast suspension.
E, compressed yeast cells only.
F, compressed yeast suspended at 25–30°C., and with supernatants from group B.

Gas Production Studies. The results of gas production studies of straight doughs, summarized in Table VI, help to explain the poor loaf volumes obtained when ADY was rehydrated at temperatures below 30° and above 40°C. Marked decreases in gas production occurred when temperatures above or below this optimum range were used in rehydrating the ADY (series A). When the variously rehydrated ADY suspensions were centrifuged and the cells alone used to prepare doughs, an even greater decrease in gas production occurred (series B). The supernatants thus obtained, however, had no depressant effect on gas production when incorporated into doughs with optimally prepared ADY (series C). As would be expected from the baking experiments, the gas production of compressed yeast doughs was decreased only by suspending it at 50°C. (series D); this may be attributed to thermal killing of some of the cells. When optimally suspended compressed yeast was centrifuged and resuspended in supernatants obtained by rehydration of ADY at various temperatures (obtained from preparation of series B) and incorporated into doughs, the results shown in series F were obtained. No effect on gas production was noted except with the supernatants obtained at 5° and 10°C., in which marked stimulation was observed along with production of sticky doughs. When the compressed yeast cells obtained by suspension at various temperatures were centrifuged and used alone in doughs (series E), however, there appeared to be a slight decrease in gas production by the cells suspended at 5° and 10°C.

The hourly rates of gas production in doughs made with optimally (40°C.) prepared ADY and compressed yeast are shown in Fig. 2,

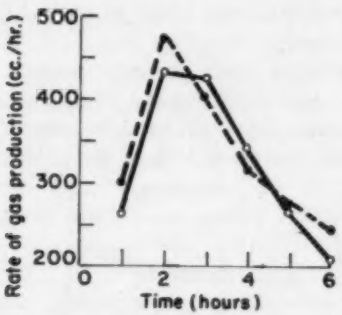

Fig. 2. Rate of gas production in straight doughs containing compressed yeast (open circles) or active dry yeast (solid circles). The ADY was rehydrated at 40°C. for 20 minutes.

which gives the gas production of a straight dough for 6 hours; it is quite obvious that the two yeasts are very similar in rate of gas production, although at the 5% level of significance ADY exceeded the compressed yeast during the first, second, and sixth hours, with the reverse at the third and fourth hour.

Figure 3, which compares the rate of gas production by ADY and compressed yeast during sponge and dough fermentation, shows that although the compressed yeast sponge produced gas at a faster rate during the first 2 hours, the reverse was true for the third and fourth hours of sponge fermentation. During the fourth hour of sponge fermentation the ADY sponge and the compressed yeast sponge produced 436 and 247 cc. respectively. The totals for the sponge

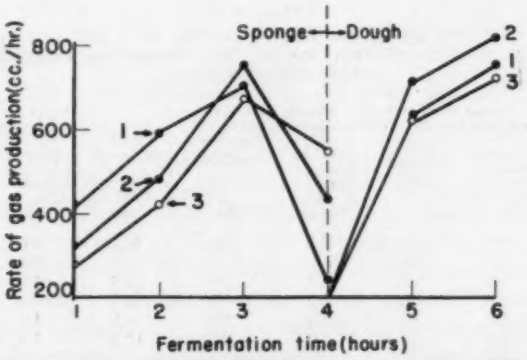

Fig. 3. Rate of gas production in sponge and doughs containing: 1), 2.25% compressed yeast; 2), 1.13% ADY; 3), 0.90% ADY. The ADY was rehydrated at 40°C. for 20 minutes.

fermentation were 1,998 and 1,968 cc. for ADY and compressed yeast doughs respectively.

Since about the same total quantity of carbon dioxide was produced during this period, it would appear that sugar had been depleted to the same extent in both sponges. During the 2-hour dough fermentation, however, 10% more gas was produced by the ADY dough than by the compressed yeast dough. The total gas production for the entire 6-hour sponge and dough fermentations was 3,542 and 3,362 cc. for ADY and compressed yeast respectively; a difference which was not significant.

Farinograph Studies. Farinograph studies were made of doughs containing compressed yeast extracts and ADY extracts obtained by suspension of the yeasts at various temperatures, and also with glutathione in quantities equal to those leached from ADY at the various temperatures used.

Table VII summarizes the results and illustrates the marked similarity between doughs prepared with ADY rehydrated at various temperatures and those with added glutathione. Compressed yeast lost a very small amount of material at all temperatures and, as expected, supernatants from its suspension had no effect on the farinograms. ADY extracts obtained by rehydration of the yeast at temperatures below 30°C. greatly shortened dough development time and lowered the mixing tolerance. Very comparable results were obtained with added glutathione.

Figure 4 illustrates the marked effect of ADY extracts and glutathione on farinograph curves. At all temperatures of rehydration,

TABLE VII

INFLUENCE OF ACTIVE DRY YEAST AND COMPRESSED YEAST EXTRACTS PREPARED AT DIFFERENT TEMPERATURES, AND OF GLUTATHIONE, ON FARINOGRAM CHARACTERISTICS OF FLOUR

Yeast Suspension Temp.	Doughs Made with Compressed Yeast Extracts[a]		Doughs Made with ADY Extracts[b]		GSH per Dough	Doughs Made with Glutathione[b]	
	Dough Development Time[c]	"Drop-off" Value[d]	Dough Development Time[c]	"Drop-Off" Value[d]		Dough Development Time[c]	"Drop-Off" Value[d]
°C	*minutes*	*B.u.*	*minutes*	*B.u.*	*mg*	*minutes*	*B.u.*
5	5.0	60	3.0	100	41.0	3.0	130
10	4.5	60	3.5	110	32.9	3.5	125
20	5.0	55	4.0	90	23.4	4.0	100
30	5.0	55	5.0	85	9.0	4.5	90
40	5.0	55	5.0	80	9.0	4.5	90
50	5.0	55	5.0	90	14.0	4.0	96

[a] Extracts of 1.5% ADY and 3.0% compressed yeast (flour basis) respectively.
[b] Glutathione used in amounts equivalent to that leached from ADY at the temperatures indicated.
[c] Time in minutes for dough to reach maximum peak of 500 B.u.
[d] Decrease in dough consistency from maximum after 20 minutes of mixing.

the doughs containing compressed yeast extracts gave curves which remained on the 500 B.u. line for approximately 10 minutes and were identical to a control curve (not shown). The curves obtained using doughs containing ADY extracts dropped almost immediately after reaching the 500 B.u. line. As the temperature of rehydration was lowered from 40°C. the decrease in consistency became progressively more rapid.

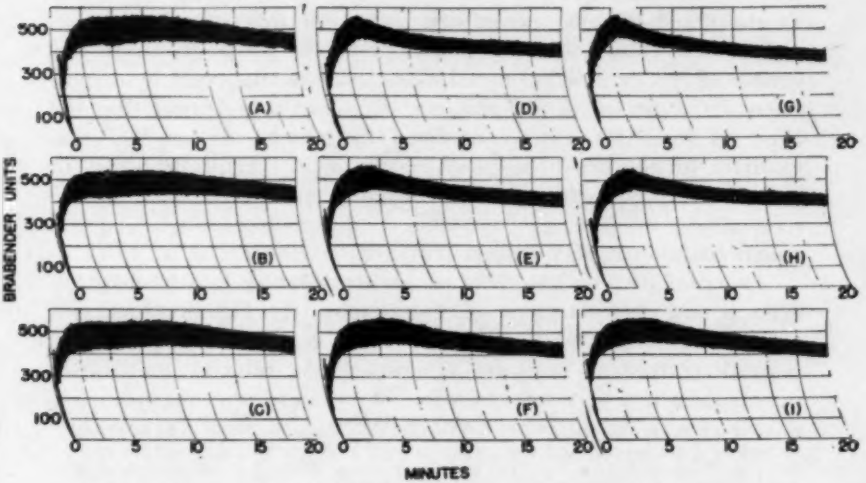

Fig. 4. Farinograms obtained on flour-water-salt doughs containing supernatants of compressed yeast, of ADY, or glutathione.

Compressed yeast supernatants suspended °C.	ADY supernatants rehydrated °C.	Glutathione added mg
A 5	D 5	G 41.0
B 20	E 20	H 23.4
C 40	F 40	I 9.0

Extensigraph Studies. The extensigrams were analyzed by the method described by Dempster *et al.* (3,4). The load, in grams, supported by the dough at a constant deformation of 8 cm. plotted against rest period gave an indication of the extent of relaxation, with time, which the dough had undergone; the curves approximated rectangular hyperbolas. By plotting load times rest period against rest period, straight lines resulted, the intercepts and slopes of which changed with reaction time. The intercepts or "relaxation constants" are a measure of the rate at which a dough relaxes. A large relaxation constant for a dough indicates a "tighter" dough which takes longer to relax than one with a smaller relaxation constant.

When ADY was rehydrated at temperatures below 30°C., a marked increase in extensibility of the doughs, as judged by their handling qualities, had been observed. This effect is shown by the relaxation curves in Fig. 5. Even when ADY was rehydrated at 40°C., a marked softening effect of the extract on the dough occurred (curve 2) when compared with a control dough (curve 1). This was particularly noticeable at rest periods shorter than 40 minutes. As the rehydration temperature was lowered to 20° (curve 3) and 5°C. (curve 4), the dough became exceedingly extensible, offering virtually no resistance to extension. The load supported by the dough given rest periods of less than 40 minutes approached that supported by doughs given a rest period of 120 minutes. It would thus appear that the effect of the ADY extract obtained by rehydration at 5°C. was virtually to eliminate the "high-energy state" condition found in normal newly mixed doughs and causing it to appear in the relaxed state almost at once, as compared to the control dough and the dough containing ADY extract obtained by rehydration at 40°C.

The similarity of the effect of low-temperature rehydrated ADY extracts and of glutathione (GSH) is shown in Fig. 6, which is a plot of relaxation constants versus reaction time. Since a higher relaxation constant indicates a more slowly relaxing and hence tighter dough, a very marked effect of bromate with reaction time is at once seen on the control dough. As reaction time was increased

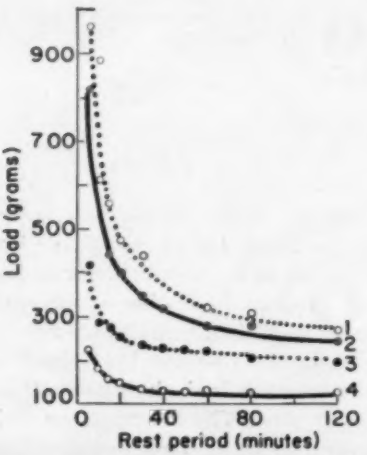

Fig. 5. Structural relaxation in flour-water-salt doughs containing: 1), no addition; 2), supernatant of 1.5% active dry yeast (flour basis) rehydrated at 40°C., 3) at 20°C., 4) at 5°C. Doughs had been allowed 5 minutes' reaction time.

in the unbromated control, the relaxation constant slowly decreased, whereas in the bromated dough (10 p.p.m.) a very great increase in relaxation constant occurred. In contrast, the relaxation constants of the doughs containing 15.6 mg. GSH or ADY extracts prepared at 20°C. remained very low and were virtually identical through-

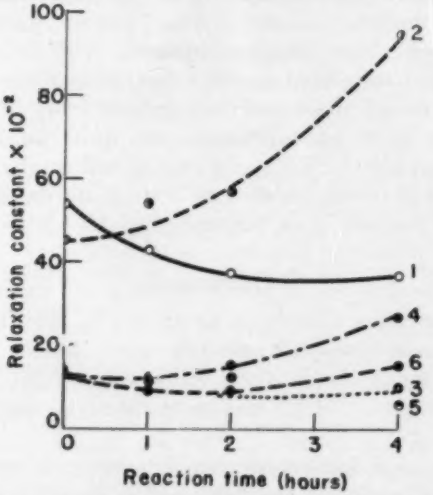

Fig. 6. The effect of active dry yeast (ADY) extracts, potassium bromate, or glutathione on the relaxation constants of doughs. 1) Control; 2) control + 10 p.p.m. potassium bromate; 3) ADY extract prepared at 20°C.; 4) ADY extracts prepared at 20°C. + 10 p.p.m. potassium bromate; 5) 15.6 mg. glutathione; 6) 15.6 mg. glutathione + 10 p.p.m. potassium bromate.

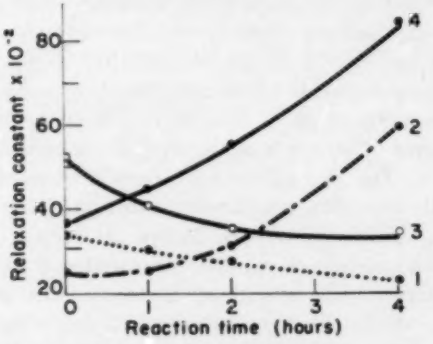

Fig. 7. The effect of active dry yeast (ADY) and compressed yeast extracts on the relaxation constants of doughs. 1) ADY extracts prepared at 40°C.; 2) ADY extracts prepared at 40°C. + 10 p.p.m. potassium bromate; 3) compressed yeast extracts prepared at 30°C.; 4) compressed yeast extracts prepared at 30°C. + 10 p.p.m. potassium bromate.

out the 4-hour reaction times. They were only slightly increased by the addition of 10 p.p.m. potassium bromate with the dough containing ADY extract responding somewhat more than that containing GSH.

The effect of ADY extract, prepared at 40°C., as compared with that of compressed yeast extract, upon the relaxation constants of flour-water-salt doughs is shown in Fig. 7. Again, even at a reaction time of 5 minutes, the dough containing ADY was in a greatly relaxed state and continued to relax further at a rate quite similar to that of the dough made with compressed yeast. The response of the two doughs to 10 p.p.m. bromate was quite similar; the relaxation constants of the ADY doughs were about one-half those of the dough made with compressed yeast during the entire "tightening-up" process as reaction time was increased to 4 hours.

Discussion

These studies show that the temperature of rehydration of active dry yeast exerts a profound influence upon doughs prepared with this leavening agent. These effects are at a minimum when rehydration is carried out at 40°C., the temperature recommended by the manufacturers.

The influence of low rehydration temperatures on doughs made with ADY is twofold: 1) the leaching of glutathione (and other substances) from the yeast cells which has a marked effect on the rheological properties of the doughs; and 2) a decrease in the fermentative power of the yeast as a result of the loss of material from the cells and an accompanying decrease in bread quality.

The result of the first effect is manifested by a shorter mixing time, a marked increase in dough extensibility, and a greater oxidizing improver requirement to obtain the best bread. The higher bromate requirement of ADY doughs results in a greater tolerance to oxidizing agents. The result of the second is a considerable decrease in bread quality. The two effects have been demonstrated by separation of the cells from the rehydration medium. The cells, when incorporated into doughs, produced loaves of inferior volume, grain, and texture. Gas production studies indicated that this was a result of decreased fermentative ability of the yeast. On the other hand, the rehydration medium when incorporated into doughs containing compressed yeast or optimally prepared ADY markedly influenced the rheological properties of the doughs, effects which could be duplicated by the addition of glutathione.

The various criteria used in these laboratory studies showed that

ADY, when rehydrated at the recommended temperature of 40°C., yielded satisfactory bread. Compressed yeast, unlike ADY, was completely unaffected by the temperature of the suspending water over a range of from 5° to 40°C. At 50°C., however, a considerable amount of inactivation occurred, with a resulting decrease in gas production and loaf volume when used in baking.

Acknowledgment

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Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minn. (1957).
2. Association of Official Agricultural Chemists. Official methods of analysis (8th ed.). The Association: Washington, D. C. (1955).
3. Dempster, C. J., Cunningham, D. K., Fisher, M. H., Hlynka, I., and Anderson, J. A. Comparative study of the improving action of bromate and iodate by baking data, rheological measurements, and chemical analyses. Cereal Chem. **33:** 221–239 (1956).
4. Dempster, C. J., Hlynka, I., and Winkler, C. A. Quantitative extensograph studies of relaxation of internal stresses in nonfermenting bromated and unbromated doughs. Cereal Chem. **29:** 39–53 (1952).
5. Herrera, T., Peterson, W. H., Cooper, E. J., and Peppler, H. J. Loss of cell constituents on reconstitution of active dry yeast. Arch. Biochem. Biophys. **63:** 131–143 (1956).
6. Hlynka, I. Structural relaxation in dough. Baker's Dig. **29:** 27–30 (1955).
7. Hlynka, I., and Anderson, J. A. Laboratory dough mixer with an air-tight bowl. Cereal Chem. **32:** 83–87 (1955).
8. Jorgensen, H. J. R. Studies on the nature of the bromate effect. Oxford University Press: London (1945).
9. Kent-Jones, D. W., and Amos, A. J. Modern cereal chemistry (5th ed.). Northern Pub. Co., Ltd.: Liverpool (1957).
10. Peppler, H. J., and Rudert, F. J. Comparative evaluation of some methods for estimation of the quality of active dry yeast. Cereal Chem. **30:** 146–152 (1953).
11. Sant, R. K., and Peterson, W. H. Factors affecting loss of nitrogen and fermenting power of rehydrated active dry yeast. Food Technol. **12:** 359–362 (1958).
12. Woodward, Gladys E., and Frey, Edith G. The determination of blood glutathione. J. Biol. Chem. **97:** 465–482 (1932).

STUDIES WITH RADIOACTIVE TRACERS

V. The Reduction of S^{35}-Labeled Persulfate to Sulfate in Flour and Dough Systems[1]

C. C. Lee and D. G. Small

ABSTRACT

S^{35}-labeled persulfate is reduced completely to sulfate by water-flour slurries in about 60 minutes or by water-flour doughs in 15 to 30 minutes. Gluten slurries can also reduce some persulfate to sulfate, but other flour constituents may also take part in reactions with persulfate. Simple extraction of slurry or dough with water alone appears capable of extracting more sulfate than persulfate. A more efficient extraction resulted in the recovery of greater amounts of persulfate. Apparently, persulfate is more strongly adsorbed. In sharp contrast to the behavior of bromate, defatting of the flour by extraction with petroleum ether or with water-saturated *n*-butanol showed hardly any significant influence on the rapid decomposition of persulfate by doughs prepared from ordinary or defatted flour.

In previous work in this laboratory with radioactive tracers, information obtained from studies with Br^{82}-labeled bromate in flour and dough systems have been reported (6,7,8). An extension of these studies included investigations using S^{35}-labeled persulfate. Although persulfate, as measured by a fluorometric method (1), has been found to decompose rapidly in flour doughs, a more detailed examination of persulfate decomposition in flour and dough systems by means of the sensitive tracer technique is of interest. This paper reports results obtained on the conversion of S^{35}-labeled persulfate to sulfate in flour or gluten slurries and in doughs made from ordinary or defatted flour.

Materials and Methods

S^{35}-Labeled Potassium Persulfate.[2] Potassium persulfate-S^{35} was prepared from potassium sulfate-S^{35} by electrolytic oxidation (4,10). The electrolytic cell was a 20-ml. test tube into which the electrodes were placed. The electrodes consisted of two smooth platinum wires, 12 mm. long and 0.4 mm in diameter, sealed onto a piece of Pyrex tubing. During electrolysis, the anode extended straight downward nearly to the bottom of the cell while the cathode was looped around the Pyrex tubing above the anode near the surface of the electrolyte (4). Immediately before an electrolysis, the electrodes were carefully heated until glowing to ensure the efficiency of a smooth anode (5).

[1] Manuscript received July 24, 1959. Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan. For paper IV, see reference 8.

[2] This compound was prepared by R. Tkachuk.

A solution of 300 mg. reagent grade potassium sulfate and 1.5 mg. potassium ferrocyanide in 3.75 ml. of 1*N* sulfuric acid containing 5 mc. S^{35} was placed in the cell. The inclusion of the ferrocyanide has been reported to give better yields (5). The electrodes were then immersed into the electrolyte and electrolysis was carried out for 15 hours with a current of 200 to 210 milliamperes at a potential of 6.5 to 7.0 volts. The temperature of the electrolyte was kept at 12 to 17°C. during the electrolysis by cooling the cell in an ice bath.

After the electrolytic oxidation, the resulting mixture was cooled to just above its freezing point. The crystalline product was filtered off and washed with ice-cold water. To this product, 1.00 g. of ordinary potassium persulfate and 200 mg. of ordinary potassium sulfate were added; the latter served as hold-back carrier to aid the purification. The mixture was rapidly recrystallized twice from water at 90°C. The dried product weighed 1.076 g. The radiochemical yield, based on the S^{35} activity recovered in the product, was 72%. The radiochemical purity of the product was found to be greater than 99.5% persulfate.

Flour and Freeze-Dried Gluten. The flour used in all experiments was an untreated, commercially milled, patent flour. Its protein and ash contents, on 14% moisture basis, were 12.4% (N × 5.7) and 0.35%, respectively. The freeze-dried gluten was prepared from this flour according to the method of Lusena (9).

Methods for Water Slurries of Flour or Freeze-Dried Gluten. Ten grams of flour (at 14% moisture) and 100 ml. of distilled water, containing S^{35}-labeled potassium persulfate amounting to 100, 200, 300, 400, or 1000 p.p.m. based on the weight of flour, were stirred in a Waring Blendor at high speed for 5, 15, 30, or 60 minutes. The resulting mixture was centrifuged and the clear supernatant liquid, to be called the simple aqueous extract, was used for determinations of total recovery of S^{35} and of conversion of persulfate to sulfate.

The recoveries of S^{35} in the simple aqueous extracts described above were relatively low. In another set of experiments with water-flour slurries, the extraction procedure was modified so as to give greater S^{35} recoveries. The method adopted was essentially that of Auerbach and co-workers (1), with the exception that zinc chloride was used in place of zinc sulfate. One gram of flour and 34 ml. of distilled water containing 100, 200, 300, 400, or 1000 p.p.m. potassium persulfate-S^{35} based on the weight of flour were shaken for 5 minutes by a mechanical shaker. Four milliliters of 0.3*N* zinc chloride, 12 ml. of 0.1*N* sodium hydroxide, and 0.5 g. of Celite filter aid were then added and the mixture shaken for an additional 15-minute period.

After centrifugation, the supernatant liquid, to be called the zinc hydroxide-clarified extract, was used for activity assays.

In the studies with freeze-dried gluten slurries, 1.0 g. of the gluten and 34 ml. of distilled water containing 100, 200, 300, 400, or 1,000 p.p.m. labeled postassium persulfate based on the weight of gluten were shaken for 5 minutes. The resulting mixture was worked up to give the zinc hydroxide-clarified extract exactly as described for the water-flour slurries.

Methods for Doughs. Nonfermenting doughs were prepared from 100 g. (at 14% moisture) of ordinary flour, flour defatted by extraction with petroleum ether, b.p. 40 to 60°C., or flour defatted by extraction with water-saturated *n*-butanol (11), 60 ml. of distilled water, and 100, 200, 300, 400, or 1,000 p.p.m. S^{35}-labeled potassium persulfate. The fat removed by extraction with petroleum ether and with water-saturated *n*-butanol were, respectively, 0.69 and 1.47% of the weight of flour. Each of these doughs was mixed for 3 minutes in a Hobart mixer and then placed in a cabinet maintained at 30°C. Suitable subsamples were removed at 0, 15, and 30 minutes after mixing and worked up to give either simple aqueous extracts or zinc hydroxide-clarified extracts of the dough.

Simple aqueous extracts were obtained from doughs from ordinary and petroleum ether-extracted flours by dispersing a 20-g. subsample in 100 ml. of distilled water in a Waring Blendor operated at high speed for 5 minutes. Zinc hydroxide-clarified extracts were obtained from doughs from ordinary, petroleum ether-extracted, and water-saturated *n*-butanol-extracted flours. In each extraction, a 2.0-g. subsample of dough was dispersed in 68 ml. of distilled water, 8 ml. of 0.3*N* zinc chloride, 24 ml. of 0.1*N* sodium hydroxide and 1.0 g. of Celite in a Waring Blendor operated at high speed for 5 minutes.

Determinations of Sulfate and Persulfate. Aqueous extracts from flour slurries, gluten slurries, or doughs were assayed for sulfate and persulfate by the removal of the sulfate as insoluble barium sulfate (3,12). Suitable quantities of inactive sulfate as carrier and inactive persulfate as hold-back carrier were added before the precipitation of the barium sulfate. Since aqueous extracts from these experiments may contain both sulfate-S^{35} and persulfate-S^{35}, the accuracy of the actual experimental procedure was tested by trial assays with known mixtures of sulfate-S^{35} and persulfate-S^{35}. For example, with a mixture of 20% sulfate-S^{35} and 80% persulfate-S^{35}, after removal of barium sulfate, the activity remaining in solution amounted to 79.5 and 80.2% for duplicate trials.

Corrections for radioactive decay in the counting of all S^{35} samples

were made in the following way: Immediately before and after the counting of any given sample, a sample containing a fixed amount of S^{35}, to be called the standard, was also counted. The S^{35} activity of the standard used in all the present experiments was 1,000 c.p.m. at an arbitrarily fixed zero time. If the activity of a given sample were n c.p.m. and the mean activity of the standard counted before and after the counting of the sample were, for example, 900 c.p.m., then the activity of the sample corrected to zero time would be

$$\frac{1000}{900} \times \text{n c.p.m.}$$

Results and Discussion

As an illustration of the actual experimental results and calculations, a description is given for the determination of S^{35} recovery and sulfate content in one of the duplicate experiments with the simple aqueous extract from the flour slurry which was stirred in the Waring Blendor for 5 minutes with 100 p.p.m. labeled potassium persulfate. To 40 ml. of this extract, 1.0 ml. of a solution of ordinary potassium persulfate (27 mg. or 0.1 mmole per ml.) and 1.0 ml. of a solution of ordinary potassium sulfate (27 mg. or 0.15 mmole per ml.) were added as carriers. Two-milliliter aliquots were then dried in aluminum pans and counted. The mean activity, corrected to zero time, was 1,940 c.p.m.

The total volume of aqueous extract, including the water from the 14% moisture in the flour, was 101.4 ml. Hence the total recovery of S^{35} in the simple aqueous extract was $(101.4/2) \times (42/40) \times 1{,}940 = 103{,}300$ c.p.m.

To determine the total S^{35} activity orginally added to the flour slurry, a solution was prepared by adding a known weight of potassium persulfate-S^{35} to an aqueous extract from nonradioactive slurry obtained exactly in the same way as in the experiments with slurries containing labeled persulfate. The counting of dried aliquots of such a known solution gave 202,500 c.p.m. as the activity, corrected to zero time, for the 1.0 mg. of potassium persulfate-S^{35} used in this 100-p.p.m. experiment.

Therefore, the recovery of S^{35} in this sample aqueous extract was $(103{,}300/202{,}500) \times 100 = 51\%$.

For the estimation of the sulfate content in this aqueous extract, 20 ml. of the 42 ml. of extract plus carriers were treated with 1.0 ml. of a solution of barium chloride (40 mg. or 0.19 mmole per ml.). The precipitated barium sulfate was removed by centrifuging and 2.0-ml. aliquots of the supernatant liquid dried and counted. The

mean activity, corrected to zero time, was 790 c.p.m.

Therefore, the total S^{35} activity remaining after removal of sulfate was $790 \times (21/20) \times (101.4/2) \times (42/40) = 44{,}100$ c.p.m.

Since the total S^{35} recovered in the extract was 103,300 c.p.m., the sulfate content was $(103{,}300 - 44{,}100/103{,}300) \times 100 = 57\%$ of the recovered S^{35}.

In an analogous manner, the S^{35} recoveries and sulfate contents in all the extracts from the slurries and doughs were determined. The mean results from duplicate experiments are tabulated in Tables I to V.

Discussion of Results from Studies with Slurries. For water-flour slurries, conversion of persulfate to sulfate was apparently complete after a stirring time of about 60 minutes (Table I). The recoveries of S^{35} in the simple aqueous extracts of flour slurries ranged between 51 and 68% of the persulfate initially added (Table I). Some 30 to 50% of the initially added persulfate was thus lost from the aqueous phase,

TABLE I

RECOVERY OF S^{35} AND CONVERSION OF PERSULFATE TO SULFATE IN SIMPLE AQUEOUS EXTRACTS FROM WATER-FLOUR SLURRIES

Initial Persulfate (I.P.) Level	Stirring Time (Minutes)							
	5		15		30		60	
	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}
ppm	*%I.P.*	%	*%I.P.*	%	*%I.P.*	%	*%I.P.*	%
100	52	57	64	82	64	97	57	100
200	53	56	61	77	60	97	62	100
300	54	50	58	75	60	98	61	100
400	56	51	59	77	56	97	58	100
1000	60	37	68	75	66	94	65	99

TABLE II

RECOVERY OF S^{35} AND CONVERSION OF PERSULFATE TO SULFATE IN ZINC HYDROXIDE-CLARIFIED EXTRACTS FROM WATER SLURRIES OF FLOUR AND OF FREEZE-DRIED GLUTEN[a]

Initial Persulfate (I.P.) Level	S^{35} Recovery in Extract		Sulfate Content of Recovered S^{35}	
	Flour Slurry	Gluten Slurry	Flour Slurry	Gluten Slurry
ppm	*%I.P.*	*%I.P.*	%	%
100	96	76	37	78
200	100	87	36	73
300	97	71	33	74
400	98	78	38	74
1000	93	77	26	71

[a] The slurries were worked up after 1.0 g. of flour or 1.0 g. of freeze-dried gluten was shaken in 34 ml. of distilled water for 5 minutes.

probably through adsorption by the flour. There is, however, no apparent relationship between the degrees of S^{35} recovery and experimental variables such as initial persulfate level or length of stirring time. When the extraction of the flour slurry was effected by the method involving clarification with zinc hydroxide, recovery of all S^{35} in the aqueous phase was nearly complete (Table II).

It may be of interest to note that the sulfate contents in the zinc hydroxide-clarified extracts of flour slurries were some 10 to 20% less than the sulfate contents in the corresponding simple aqueous extracts of flour slurries that had been stirred for only 5 minutes (compare Tables I and II). This finding appears to indicate that simple extraction with water alone may remove more sulfate than persulfate, or in other words, persulfate may be more strongly adsorbed than sulfate. Another factor which probably contributed to the lower sulfate contents in the zinc hydroxide-clarified extracts may be regarded as a concentration effect. It will be recalled that in obtaining a simple aqueous extract of flour slurry, a total of 100 ml. of water was used on 10 g. of flour, while in obtaining the zinc hydroxide-clarified extract, the final volume was 50 ml. for 1.0 g. of flour. For a given initial persulfate level, the persulfate concentration in the aqueous phase is, therefore, higher under the conditions where the simple aqueous extract was obtained. Such a higher concentration of a reactant may lead to faster reaction, resulting in a greater amount of conversion of persulfate to sulfate in the simple aqueous extract.

Slurries of freeze-dried gluten also reduced some persulfate to sulfate. The extents of conversion of persulfate to sulfate by 5 minutes of shaking of water slurries of 1.0-g. samples of gluten were in the neighborhood of 75% (Table II). Under similar conditions, the conversions of persulfate to sulfate by water slurries of 1.0-g. samples of flour were approximately 35% (Table II). Since only about 0.15 g. of freeze-dried gluten was obtainable from 1.0 g. of flour, the gluten in 1.0 g. of flour could probably account for $0.15 \times 75 = 11\%$ of the conversion of persulfate to sulfate, which is considerably less than the actual 35% conversion effected by 1.0 g. of flour under these conditions. Such considerations may be regarded as indicating that components of flour other than gluten also undergo oxidation-reduction reactions with persulfate. A similar conclusion was arrived at from measurements of the degrees of reduction of Br^{82}-labeled bromate to bromide by water slurries of flour and of freeze-dried gluten (8). Another explanation for the difference between gluten and flour in conversion of persulfate to sulfate may also be considered. If some de-

naturation had occurred during the preparation of the freeze-dried gluten, then it is possible that the isolate gluten would not react with as much persulfate as an equivalent amount of gluten in the original flour.

Discussion of Results from Studies with Doughs. In the studies with doughs, simple extraction of 20-g. subsamples with 100 ml. of water resulted in recoveries in the extract of only about 35 to 45% of the S^{35} initially introduced as persulfate. The S^{35} present in such simple aqueous extracts was practically all in the form of sulfate, even when the water extraction was carried out immediately after the dough was formed by 3 minutes of mixing. Similar results were observed when the dough was prepared from either ordinary or petroleum ether-extracted flour.

Extraction of 2.0-g. subsamples of dough by the more efficient method involving zinc hydroxide clarification resulted in higher S^{35} recoveries of the order of 70 to 80%. For doughs extracted immediately after mixing, some unchanged persulfate was present in the zinc hydroxide-clarified extracts, as the sulfate contents of such extracts were in the neighborhood of 80% of the recovered S^{35}. That the nonsulfate portion of the recovered S^{35} was indeed unchanged persulfate was indicated by the observation that such residual S^{35}-containing material could again be completely converted to sulfate by heating with dilute hydrochloric acid. The hydrochloric acid presumably was oxidized by the unchanged persulfate. If the doughs were allowed to rest for various lengths of time before extraction, conversion of persulfate to sulfate was practically complete when the rest time was 15 to 30 minutes. Essentially similar results were observed for doughs prepared from ordinary flour, petroleum ether-extracted flour, or water-saturated *n*-butanol-extracted flour (Tables III, IV, V).

Comparing the conversions of persulfate to sulfate in doughs and in flour slurries, it is clear that such conversions are faster in the doughs. A factor, though not necessarily the only factor, that contributes to the differences in rates of persulfate decomposition in slurries and doughs may be the effects of reactant concentration. Since the volume of water used in making up a dough is much less than the volume used for a slurry, one may regard the concentration of reactants in doughs as higher than in slurries. Thus, in a dough the reactions are faster, and consequently there is a more rapid conversion of persulfate to sulfate.

The rapid and complete reduction of persulfate to sulfate by water-flour doughs is quite different from the behavior of bromate (6).

TABLE III

RECOVERY OF S^{35} AND CONVERSION OF PERSULFATE TO SULFATE IN ZINC HYDROXIDE-CLARIFIED EXTRACTS OF DOUGHS FROM ORDINARY FLOUR

Initial Persulfate (I.P.) Level	Rest Time after Mixing of Dough (Minutes)					
	0		15		30	
	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}
ppm	*%I.P.*	%	*%I.P.*	%	*%I.P.*	%
100	72	83	74	97	83	100
200	82	78	74	96	81	100
300	77	81	76	97	81	99
400	73	81	81	98	84	100
1000	76	71	77	90	79	97

TABLE IV

RECOVERY OF S^{35} AND CONVERSION OF PERSULFATE TO SULFATE IN ZINC HYDROXIDE-CLARIFIED EXTRACTS OF DOUGHS FROM PETROLEUM ETHER-EXTRACTED FLOUR

Initial Persulfate (I.P.) Level	Rest Time after Mixing of Dough (Minutes)					
	0		15		30	
	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}
ppm	*%I.P.*	%	*%I.P.*	%	*%I.P.*	%
100	71	87	73	97	80	100
200	67	80	73	97	63	100
300	75	81	74	97	72	99
400	79	75	76	93	72	98
1000	72	68	73	88	79	96

TABLE V

RECOVERY OF S^{35} AND CONVERSION OF PERSULFATE TO SULFATE IN ZINC HYDROXIDE-CLARIFIED EXTRACTS OF DOUGHS FROM WATER-SATURATED *n*-BUTANOL-EXTRACTED FLOUR

Initial Persulfate (I.P.) Level	Rest Time after Mixing of Dough (Minutes)					
	0		15		30	
	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}	S^{35} Recovery in Extract	Sulfate Content of Recovered S^{35}
ppm	*%I.P.*	%	*%I.P.*	%	*%I.P.*	%
100	81	85	81	93	80	98
200	74	83	80	99	75	99
300	80	82	82	96	82	99
400	80	83	83	95	77	99
1000	76	69	82	91	76	97

The fact that persulfate is a more powerful oxidizing agent than bromate may at least be partly responsible for the different behavior of persulfate and bromate in flour slurries and doughs.

From the studies with doughs made from flour defatted by extraction with petroleum ether or with water-saturated *n*-butanol, the results obtained show hardly any significant differences in the rapid decomposition of persulfate by doughs from ordinary or defatted flours (Tables III to V). Such results are again in sharp contrast to the behavior of bromate. Lee and Tkachuk (7) have noted a marked decrease in the the degree of conversion of Br^{82}-labeled bromate to bromide when the dough was prepared from petroleum ether-extracted flour. Similarly, decreases in the rates of disappearance of bromate, measured by amperometric titration, were reported by Cunningham and Hlynka (2) for doughs prepared from flours defatted by extraction with petroleum ether or water-saturated *n*-butanol. Thus flour lipids appear to be involved, either directly or indirectly, in the oxidation-reduction reactions of bromate in dough systems. With persulfate, on the other hand, the flour lipids do not appear to exert any appreciable effect on its rapid decomposition by the dough.

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Literature Cited

1. Auerbach, M. E., Eckert, H. W., and Angell, Eleanor. The determination of persulfate in flour and dough. Cereal Chem. **26:** 490–496 (1949).
2. Cunningham, D. K., and Hlynka, I. Flour lipids and the bromate reaction. Cereal Chem. **35:** 401–410 (1958).
3. Eager, R. L., and McCallum, K. J. The mechanism of persulfate oxidations. Can. J. Chem. **32:** 692–695 (1954).
4. Elbs, K. Electrolytic preparations: exercises for use in the laboratory by chemists and electrochemists (trans. by R. S. Hutton), pp. 35–40. Edward Arnold: London (1903).
5. Glasstone, S., and Hickling, A. Electrolytic oxidation and reduction: inorganic and organic, pp. 265–266. Chapman and Hall: London (1936).
6. Lee, C. C., Tkachuk, R., and Finlayson, A. J. The conversion of Br^{82}-labeled bromate to bromide in nonfermenting and fermenting doughs and in baked bread. Cereal Chem. **35:** 337–349 (1958).
7. Lee, C. C., and Tkachuk, R. Studies with radioactive tracers. III. The effects of defatting and of benzoyl peroxide on the decomposition of Br^{82}-labeled bromate by water-flour doughs. Cereal Chem. **36:** 412–420 (1959).
8. Lee, C. C., and Tkachuk, R. Studies with radioactive tracers. IV. Degrees of reduction of Br^{82}-labeled bromate to bromide by some components of flour. Cereal Chem. **37:** 228–233 (1960).
9. Lusena, C. V. Preparation of dried native wheat gluten. Cereal Chem. **27:** 167–178 (1950).
10. Marshall, H. The persulfates. J. Chem. Soc. **59:** 771–786 (1891).
11. Mecham, D. K., and Mohammad, A. Extraction of lipids from wheat products. Cereal Chem. **32:** 405–415 (1955).
12. Riesebos, P. C., and Aten, A. H. W., Jr. Absence of rapid exchange of sulfur atoms between sulfate and persulfate ions. J. Am. Chem. Soc. **74:** 2440 (1952).

FIVE PROPOSED METHODS FOR DETERMINING SMUT CONTENT IN WHEAT[1]

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ABSTRACT

Five simple, uniform, and accurate methods for quantitative determination of the total smut contamination in wheat have been developed; namely: light transmittance, sedimentation, catalase activity, light reflectance, and light absorption. These methods were applied to 138 samples of smutty wheat ranging from 0.5 to 3.0% smut and representing three crop years. The results were compared to smut spore counts based on a reference microscopic method. Correlation coefficients for the relation between test values for all five methods and respective spore counts were highly significant.

Six factors – namely, simplicity, practicability, rapidity, accuracy, precision, and cost of equipment – were considered in evaluating the methods. The sedimentation method would be preferred where low cost of operation is desired. This method could also be used in areas of low smut incidence, where an occasional test for smut may be required. The sedimentation method was used to differentiate between smut and other forms of mold infection in samples of questionable smutty wheat. However, the light-absorption method measuring smut-spore concentration directly on bulk wheat, which takes only 45 seconds per determination, was selected as the most practical and suitable method for routine testing of smut content in wheat where time is a prime factor.

The objective of this research was to develop a uniform, accurate method for determining the total amount of smut in wheat which may be used in routine inspection under the official grain standards of the United States. In selecting the best method the most important considerations are simplicity, practicability, rapidity, accuracy, precision, and cost of equipment.

The present methods for determining the amount of smut in wheat lack accuracy and precision and are slow. Some wheat samples grade smutty on odor alone, some on loose spores adhering to the brush ends of kernels (tagged ends), and some on smut balls alone; some samples grade smutty on a combination of two or three of these criteria. There is need for a uniform, simple, rapid, and more accurate method for quantitative determination of total smut contamination in wheat, including smut balls, broken smut balls, and smut spores.

The search for a method led to several possible approaches. Besides the classic Levi counting-chamber method by Heald (5), the

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following procedures were investigated: 1) analysis of wheat washings or 2) analysis of intact wheat by a light-absorption method. The washings were analyzed by measuring: light transmittance through the spore suspension; volume of sediment; catalase activity; or light reflected from the sediment after it was filtered from the suspension.

Materials and Methods

One hundred and thirty-eight samples obtained from the Pacific Northwest area from the 1954, 1955, and 1956 crops were used. All dockage that could be readily separated from the samples by appropriate sieves was removed. For most accurate results, damaged kernels and foreign material were also removed by hand separation. These samples were graded "smutty" by Government grain supervisors according to the smut dockage method, and the samples were classified into three groups as follows:

Light smut: 0.5 but less than 1.0% smut dockage
Medium smut: 1.0 but less than 2.0% smut dockage
Heavy smut: 2.0% or more smut dockage

Reference Microscopic Method

Cherewick (3) described the use of a wetting agent to aid in removing spores from grain, and the use of a gelatin solution to suspend them so that they would not settle out. In this research, Bactoagar (0.1% solution) was added to the wash water instead of gelatin. A 0.1% solution of a wetting agent (Tween-20, a product of Atlas Powder Co.)[3] was also added to the warm wash water to facilitate removal of spores from the brush ends of the kernels. The presence of wheat starch grains in the filtered wash-water suspensions interfered with smut-spore counts, since the size and shape of the starch grains and colorless, sterile smut spores were frequently similar. Boiling the water suspensions for approximately 30 seconds solubilized the starch grains but left the thick-walled smut spores intact. After cooling to approximately 50°C., about 0.1 g. of Takadiastase was added to eliminate the starch grains.

The microscopic method described below, which includes the above modifications and additions to Cherewick's method, was used as a basis for checking the five proposed methods for determining smut-spore content. The method which was adopted is as follows:

> Wash 100 g. of dockage-free wheat with about 110 ml. of warm agar-Tween-water solution (1:1:1000) in an 8-oz. screw-capped square jar for 5 minutes, using a mechanical shaker.

[3] The mention of specific instruments or trade names is made for the purpose of identification and does not imply any endorsement by the United States Government.

Decant suspension through a 70-mesh screen and rinse screen with approximately 10 ml. of warm water.

Bring filtrate and washings (generally about 97 ml.) to the boiling point.

Add approximately 10 mg. of Takadiastase after cooling to approximately 50°C.

Make to 100 ml. in a graduated cylinder and mix.

Remove a drop of this suspension with micropipet, place on a hemocytometer, cover with a cover glass, and count spores.

All spores in all fields of six different mounts were counted and the average spore count per mount was then multiplied by a factor of one-million [100 (ml.) × 10,000 (hemocytometer factor)] to convert the results to the number of spores in the 100-g. sample of wheat.

Light Transmittance Method

Smut-water suspensions can be divided into many degrees of opacity, depending on the concentration of the spores in suspension. Light transmittance of these various suspensions was measured at a wave length of 525 mμ, because greatest differences in light transmittance between smutty and nonsmutty samples were apparent at this wave length. A Bausch & Lomb Spectronic "20" colorimeter was used to measure the light transmittance through the smut-water suspension.

To determine the relation between absorbance and spore count, the "Spectronic 20" was used to estimate the absorbance of suspensions containing different concentrations of pure smut ranging from 2 to 20 mg. per 50 ml. of agar-water solutions. Pure smut spores were prepared by crushing smut balls in a mortar and sieving through a 400-mesh screen. A linear relationship is apparent between the meter readings at 525 mμ and the concentration of pure smut spores (Fig. 1).

The group of 138 samples of smutty wheat was treated in the manner described under "Reference Microscopic Method." Absorbance readings at 525 mμ were made on washings from all of the

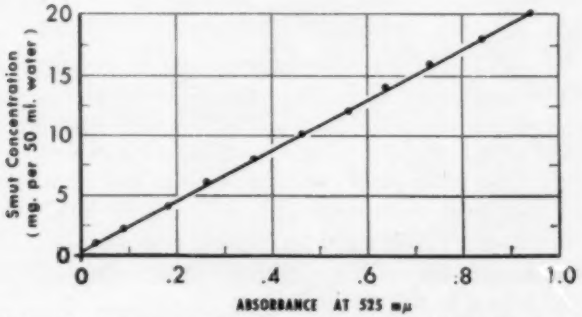

Fig. 1. Relationship between smut-spore concentrations and absorbance at 525 mμ.

samples. The results were statistically analyzed with the spore counts, and a regression equation was derived from the data. Figure 2 shows the regression equation and the straight-line relationship which exists between smut spores in suspension, obtained from washing the 138 samples of wheat, and their respective absorbance readings. The correlation coefficient for the relation between meter readings and mi-

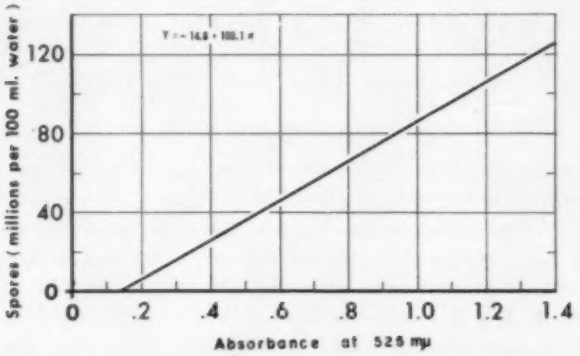

Fig. 2. Relation between smut content (Y) and absorbance (x). Y = predicted value; x = variable which is determined.

croscopic spore counts was +0.81**. The standard error of estimate in determining smut content was 0.21 million spores.

Absorbance readings corresponding to smut dockage grades are as follows:

Meter Reading	*Grade*
0.10–0.49	Light smut
0.50–1.00	Medium smut
>1.00	Heavy smut

Sedimentation Method

If it were possible for the smut spores in suspension to coalesce and form a sediment, the amount of smut might be measured directly without using an expensive instrument. Accordingly, efforts were made to find an agent which would cause sedimentation.

Among a large number of chemicals used to determine their suitability to cause spore coalescence such as Hyamine: 10X, 1622, 2389, 3500, also Tetrosan, Cetab, Ceepryn cl. and Nopcocide K, a 10% quaternary ammonium salt solution (Roccal) proved most effective and was selected for use in this method.

The proposed method is as follows:

Weigh 100 g. of wheat into a screw-capped bottle. Add approximately 120 ml. of warm tap water (120°–135°F.) and a drop of Tween-20. Allow to stand for 5 minutes. Shake vigorously for 30 seconds. Replace cap with screw-capped filter and decant into an oil centrifuge tube.

Add approximately 0.6 ml. Roccal with a pipet or graduated medicine dropper, stopper, and mix contents by inverting tube twice.

Allow to stand 10 minutes and read volume of sediment in a tapered centrifuge tube which is graduated from the bottom end.

The rate of smut-spore sedimentation after addition of Roccal is dependent on the temperature of the wash water. When a few drops

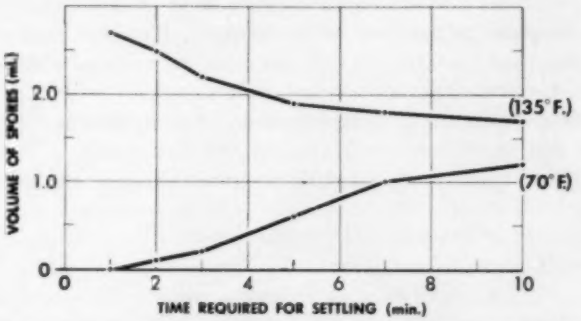

Fig. 3. Sedimentation rate as affected by temperature of suspension.

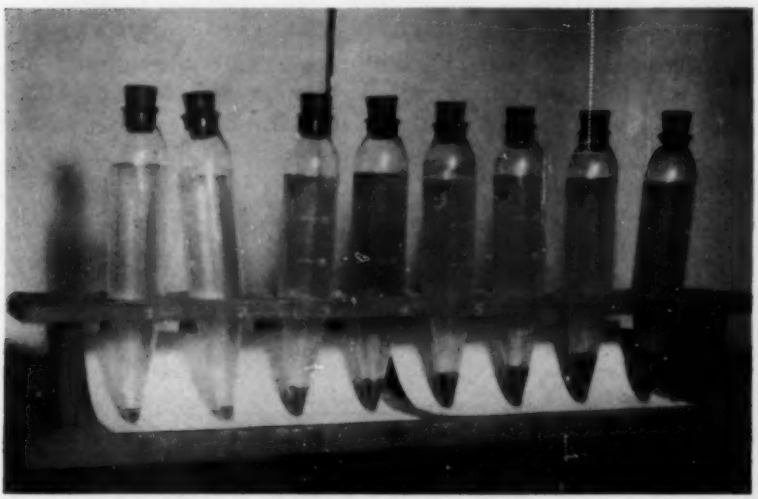

Fig. 4. Smut sediment formed 10 minutes after addition of Roccal. Tubes 1 and 2 represent no smut; 3 and 4, low; 5 and 6, medium; and 7 and 8, heavy smut.

of Roccal are added to a warm-water smut suspension, the spores begin to settle almost immediately. However, when Roccal is added to a cool-water smut suspension, sedimentation is not apparent for the first 2 minutes. Forty percent more smut can be measured at the end of 10 minutes when warm water is used than when cool water is employed for washing (Fig. 3).

In order to determine the optimum quantity of Roccal required and the time for complete sedimentation, two factors were considered: 1) rate of falling of spores, and 2) rate of compaction of the sediment.

On the basis of an experiment with three different volumes of Roccal (0.2, 0.6, and 1.2 ml.), spore falling and compaction were nearly complete at the end of 10 minutes. However, when 0.6 ml. Roccal was used the greatest differences in quantity of sediment were obtained between light and heavy smut samples.

Figure 4 shows the sediment formed 10 minutes after addition of 0.6 ml. Roccal.

Volume of spores corresponding to smut dockage grades is as follows:

Volume of spores	*Grade*
ml.	
0.5–1.49	Light smut
1.5–3.00	Medium smut
> 3.00	Heavy smut

One hundred and eleven or 80.4% of the 138 samples graded by grain supervisors using the smut dockage method fell within the proposed ranges for light, medium, and heavy smut.

Figure 5 represents the regression equation and shows the straight-line relationship that exists between results by the basic microscopic

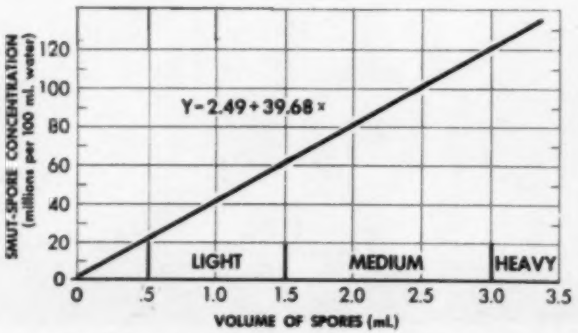

Fig. 5. Relationship between spore counts, volume of spores, and smut grades. Y = predicted value; x = variable which is determined.

and sedimentation methods. The correlation coefficient for this relationship was +0.82**. The standard error of estimate in determining smut content was 0.71 million spores.

Catalase Activity Method

The enzyme catalase is known to occur in nearly all forms of life. According to available reports in the literature, the amount of catalase in wheat decreases during ripening and is generally quite low in the harvested seed. Investigations showed that the catalase activity in smut spores is much greater than that in wheat kernels. Therefore, the catalase activity of smut water suspensions was studied for possible use as an indirect method for determining the amount of smut present in wheat.

Because there was no method for quantitative determination of catalase in smut, the method for determining catalase in beef liver described by Von Euler and Josephson (8) was modified. The amount of hydrogen peroxide that had not been decomposed after reacting with smut catalase under fixed conditions was used as a chemical measure of catalase activity in smutty wheat. The amount of hydrogen peroxide consumed in the reaction with catalase was determined by titration with potassium permanganate in an acid medium.

In the reaction between beef catalase and hydrogen peroxide, the temperature and hydrogen-ion concentration are important factors to be considered, as Morgulis *et al.* (6) have pointed out. Titrations on suspensions having different pH values and at temperatures of 10° and 30°C. indicated that this method could be carried out most satisfactorily at 10° and at a pH of 7.

Pure smut spores were prepared as before by crushing smut balls in a mortar and sieving through a 400-mesh screen. Smut-spore sus-

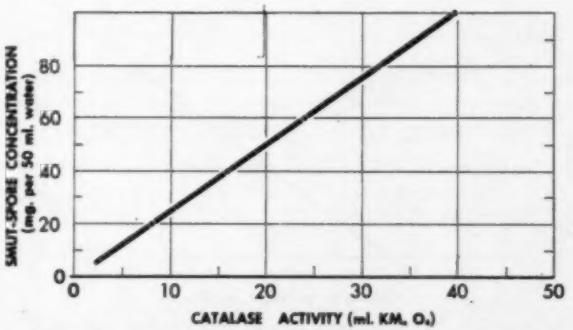

Fig. 6. Catalase activity of pure smut-spore concentration.

pensions were prepared in a pH 7 buffer. The buffer was prepared from 13.61 g. of monobasic potassium phosphate and 2.37 g. of sodium hydroxide, and made up to 2 liters. Varying concentrations of pure smut-spore suspensions, ranging from 20 to 100 mg. per 50 ml. of pH 7 buffer, were allowed to react with 40 ml. of 0.01*N* hydrogen peroxide solution for 3 hours at 10°C. The residual hydrogen peroxide was titrated with 0.01*N* potassium permanganate solution after the reaction was stopped by addition of dilute sulfuric acid solution (5%). These results are shown graphically in Fig. 6.

On the basis of these observations, a catalase activity method for determining smut content in wheat was developed.

The group of 138 officially graded smutty wheat samples, representing three crop years, was analyzed by the catalase activity procedure which follows:

> Add 60 ml. of water to 40 g. of sample, shake for 5 minutes using a mechanical shaker, decant the suspension through a 70-mesh screen, and make up to a volume of 60 ml. in a graduated cylinder. Transfer the smut suspension to a 250-ml. Erlenmeyer flask and add 40 ml. of pH 7 buffer solution and 40 ml. of the hydrogen peroxide solution. Keep the flasks in the refrigerator for 3 hours at 10°C. Stop the enzyme reaction by adding 25 ml. of dilute sulfuric acid solution and then titrate with the standard potassium permanganate solution. Make a blank determination containing all reagents except the smut suspension.

The results were expressed as the blank minus the volume of potassium permanganate solution required to react with the hydrogen peroxide remaining after reacting with the catalase present in smut. The correlation coefficient of the 138 microscopic spore counts and their respective potassium permanganate titration values was +0.75**. The line in Fig. 7 represents the regression equation. The standard error of estimate was 3.5 million spores.

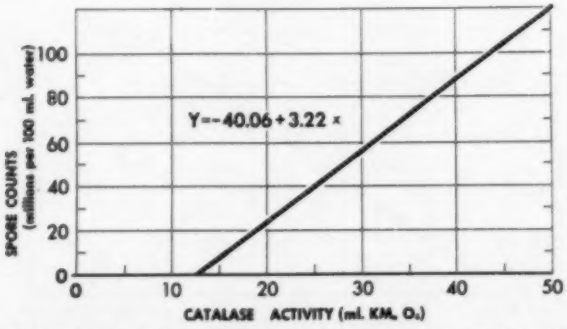

Fig. 7. Relationship between spore counts and catalase activity. Y = predicted value; x = variable which is determined.

Catalase activity values corresponding to smut dockage grades are as follows:

Catalase Activity (Blank minus titration)	*Grade*
10–23.9	Light smut
24–38	Medium smut
> 38	Heavy smut

Light Reflectance Method

Color differences in bulk wheat containing varying amounts of smut spores could not be visually differentiated in accordance with the amount of smut present on the sample, because of the natural color difference among varieties and even within a given variety of bulk wheat. However, when the smut spores were washed from wheat with water and filtered, the differences in intensity of the deposits on the filter paper were evident to the naked eye, or they could be compared visually with standard filter paper deposits or with a reflectance instrument.

An Agtron F color difference instrument (Magnuson Engineers, Inc., San Jose, Calif.) was used to measure the amount of light reflected from the particles of spores deposited on a filter paper. This reflectance meter measures only a portion of the spectrum (approximately at 540 $m\mu$). In principle, a light beam is reflected from the smut spore deposits and passes through an optical filter system to a photoelectric cell, and its intensity is indicated on a meter. Plastic color disks were selected to match the extremes in smut intensity. One of the disks was slightly darker than the heaviest smut deposit obtained from any sample, and the other disk matched the lightest smut deposit. The instrument was adjusted to give a zero reading with the dark disk and a reading of 100 with the light disk. Thus, there was a 100-point spread between lightest and heaviest smut-infected samples.

To determine the relation between the number of smut spores present on the filter paper and the light reflected from these particles, a series of samples of known smut concentrations was prepared for evaluation on the Agtron. These filter paper deposits were prepared by placing a weighed amount of pure smut spores varying from 0 to 70 mg. in 50 ml. of Tween-water solution (1:1000), shaking for 5 minutes, and decanting the smut suspension through a 70-mesh screen over filter paper. The screen was rinsed several times and the filter papers were allowed to dry for 10 minutes. The filter papers containing known smut-spore concentrations were then placed in the re-

cessed compartment of the Agtron reflectance instrument, and the readings were recorded after the meter was standardized with two appropriate color disk standards. After it was determined that pure smut-spore concentrations on filter paper and meter readings are di-

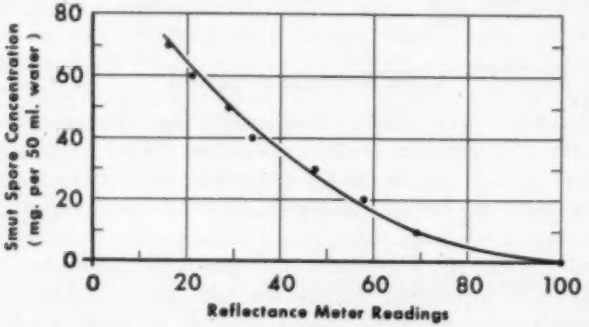

Fig. 8. Relationship between quantity of smut deposits on filter paper and reflectance meter readings.

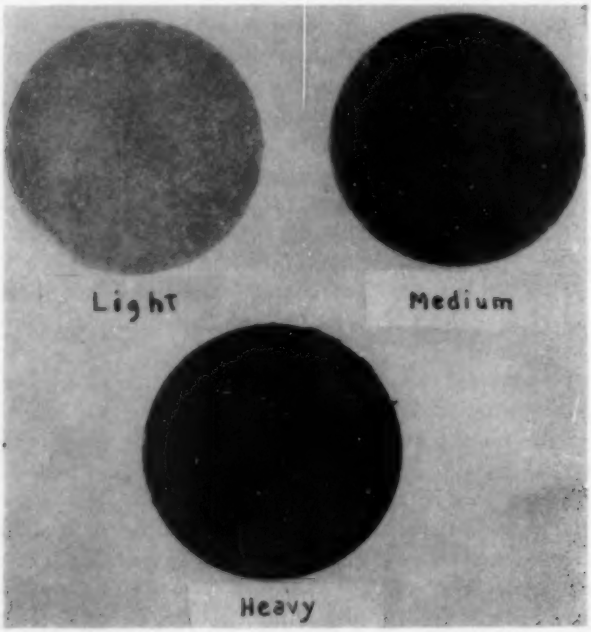

Fig. 9. Smut standards.

rectly related (Fig. 8), a method was developed for determining smut content in wheat using this technique.

Smut spores were washed from 100-g. representative portions of 138 samples of smutty wheat with 120 ml. of Tween-water solution (1:1000) and 5 minutes of agitation. The smut suspensions were decanted through a 70-mesh screen onto a 7.0-cm. no. 5 Whatman filter paper in a Buechner funnel and rinsed with several washings. The dried smut deposits on the filter paper were graded visually by comparison with three standard filter paper deposits which represented light, medium, and heavy smut infestations (Fig. 9). In this manner it was possible to grade the sample into one of the three categories in a matter of seconds. The actual microscopic spore count of 114 or 83.3% of the 138 samples visually tested was within the accepted range of the standard with which it was matched.

To evaluate the unknown sample more precisely, the 138 filter papers containing smut spores were also tested with the Agtron reflectance meter. Two plastic standards were used in the manner described previously. The individual filter papers containing spore deposits were placed in the recessed compartment and readings observed after 10 seconds.

Figure 10 shows the curvilinear relationship which exists between spore counts from the 138 wheat washings and their respective light-reflectance meter readings. The data obtained were statistically analyzed and an equation for the graph was derived. The regression equation for this relation was found to be $Y = 97.2 - 44.35 \log x$. The correlation coefficient for the relation between the meter readings and

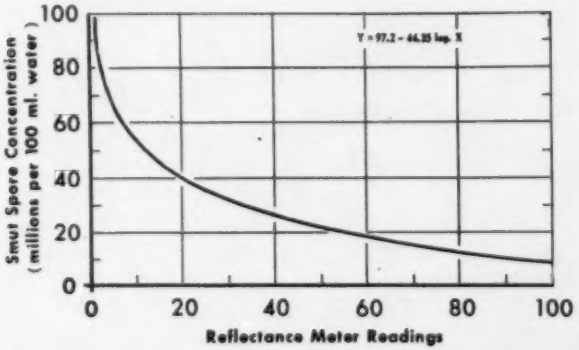

Fig. 10. Relationship between spore counts and reflectance meter readings. Y = predicted value; x = variable which is determined.

microscopic spore counts was −0.80**. The standard error of estimate was 2.3 million spores.

Reflectance meter readings corresponding to smut dockage grades are as follows:

Meter Readings	*Grade*
30.0–100	Light smut
4.0– 29.9	Medium smut
<4.0	Heavy smut

Light Absorption Method

The second general category of methods for determining the amount of smut is its measurement directly on the bulk wheat. Grain inspectors and dealers interested in a quantitative test for smut contamination on samples of wheat prefer a quick, direct test using bulk wheat rather than one in which the smut spores must be separated from the wheat before the analysis is run.

Norris (7) described a method for evaluating the internal characteristics of agricultural commodities. He used a modified Bausch & Lomb Grating Monochromator to measure the spectral transmittance curves for intact samples in the wave length range from 400 to 1000 $m\mu$. This instrument, which was later named "Rephobiospect"[4], has been shown to be applicable for the measurement of internal color of tomatoes (2) and internal characteristics of fruit (1). Measurements made on the Rephobiospect indicated that smut content in wheat could be determined by a transmittance technique, and a simpler instrument (smut meter)[5] operating on the same principle was developed and used in this investigation (Fig. 11).

The Rephobiospect consists essentially of three parts: 1) grating monochromator to illuminate the sample; 2) integrating sphere to collect the light transmitted by the sample; and 3) recording photometer to measure and record the light transmitted by the wheat sample.

Preliminary tests with the Rephobiospect showed that smutty and nonsmutty samples of bulk wheat gave characteristic differences in near infrared transmittance spectral readings. The possibility of using the differences as a basis for an objective method for estimating the degree of smuttiness in wheat was investigated. The spectral transmittance differences were observed by comparing smutty and nonsmutty wheat as follows:

An aluminum container with a clear plastic bottom, 1⅜ in. in di-

[4] This instrument is not yet commercially available. It was developed in the Agricultural Marketing Service, Marketing Research Division, Biological Sciences Branch, Quality Evaluation Section Laboratories at Beltsville, Md.

[5] This instrument is available from the American Research & Mfg. Company, Rockville, Md.

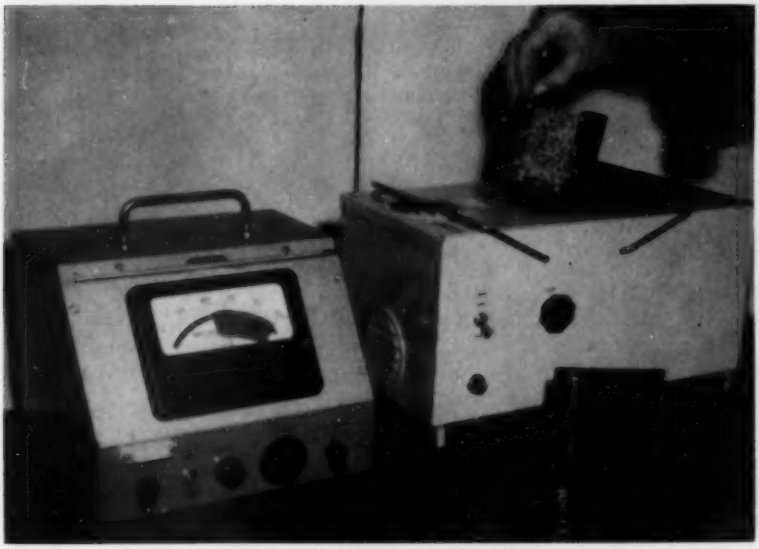

Fig. 11. Smut meter.

ameter and 1 in. in height, was filled with 15 g. of nonsmutty wheat, placed in the sample receptacle of the Rephobiospect, sphere closed, and the spectral transmittance curve recorded. Light, medium, and heavy smut-infected samples were tested in the same manner.

A less expensive portable meter (Fig. 11), which is similar in principle to the Rephobiospect, was designed and constructed for testing samples of bulk wheat for smut content in the field. This smut meter consists of two units: one unit contains the light source, filter assembly, sample holder, and a multiplier phototube; the second unit houses a meter and other electronic components. A piece of amber-colored phenolic laminated cloth (3-3/8 by 3-1/2 by 9/32 in.) was used as the standard.

The principle of operation is as follows: Light energy from a tungsten light source passes through a filter assembly, sample, and into a multiplier phototube. The absorbance difference at two wave lengths (800 and 930 $m\mu$) is read from the meter. Smut spores (dark areas) adhering to the wheat kernels increase the absorption of light at the shorter wave length relative to the longer wave length. Therefore, the greater the amount of smut in a given sample, the greater is the absorbance difference between 800 and 930 $m\mu$.

The group of 138 samples of smutty wheat was tested by the following procedure:

An unweighed sample of wheat (approximately 90 g.) is poured into a 3 by 2 by 1-10/32 in. container. The opposite faces are made of clear plastic, making the light path equal to 1-10/32 in. The container is placed in the sample compartment. The filter knob is pulled out so that the 930-mμ filter is in place, and the zero knob is adjusted so that the meter reads 0; then the filter knob is pushed in so that the 800-mμ filter is in position and the meter reading is taken.

Figure 12 shows that maximum differences between the nonsmutty wheat spectrum (curve I) and the three smutty wheat spectra (curves II, III, IV) are evident at approximately 800- and 930-mμ wave lengths. The system response is indicated by curve V.

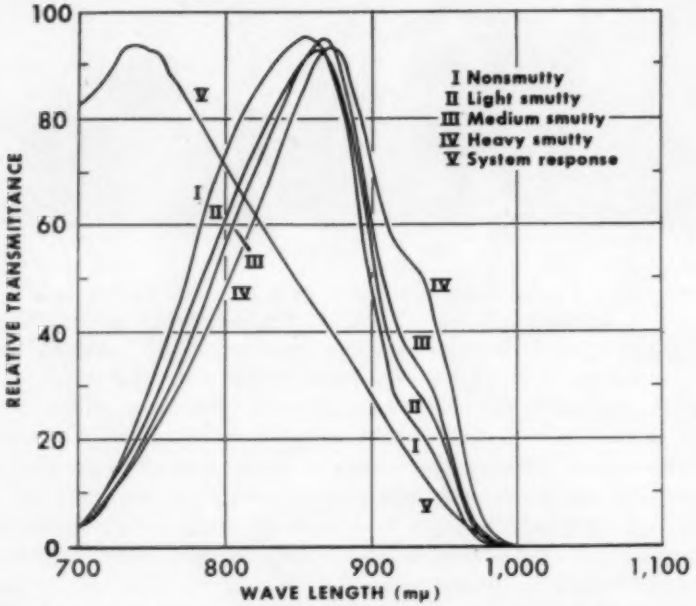

Fig. 12. Relative transmittance curves for four samples of wheat.

The presence of smut in bulk wheat is indicated by a lower relative transmittance value at 800 and a higher value at 930 mμ when compared to nonsmutty wheat samples. By measuring the transmittance at two wave lengths and using the ratio of these values as the measure of smut content, the most meaningful results were obtained. A ratio measurement gives a value which is insensitive to changes in lamp intensity, amplifier gain, size and shape of kernels, and similar factors which affect both wave lengths. A transmittance ratio[6] is equiv-

[6] Transmittance ratio = light energy at 800 mμ/light energy at 930 mμ.

alent to an absorbance difference[7] measurement. The smut meter is designed to measure absorbance differences rather than a transmittance ratio which is measured by the Rephobiospect.

One hundred and thirty-eight samples of smutty wheat were tested for smut using the smut meter. Duplicate analyses showed that the results are reproducible within 5% of the average of ten replicate determinations.

The spore counts from the basic microscopic method for determining smut in wheat were correlated with the meter readings. The curve in Fig. 13 was derived from the data. The regression equation for this relationship was found to be log $Y = 5.388 + 3.815 \log x$. With the use of this equation and plotted logs of the variables, a straight line was obtained. The correlation coefficient for the spore counts and absorption data in the near infrared region was $+0.85^{**}$. The standard error of estimate was 0.26 million spores.

The relation between meter readings and smut grades was determined and the results are shown in Table I. The smut meter read-

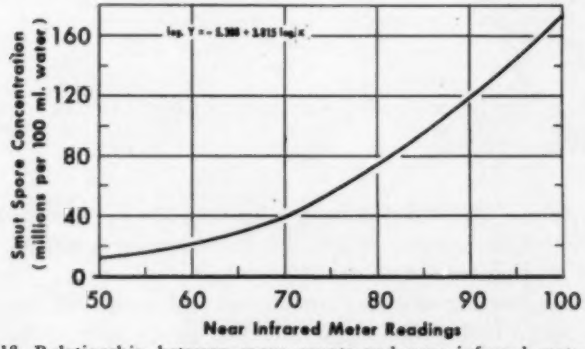

Fig. 13. Relationship between spore counts and near infrared meter readings. Y = predicted value; x = variable which is determined.

TABLE I

RELATION BETWEEN SMUT METER READINGS AND SMUT GRADES

SMUT GRADE	NO. OF SAMPLES	METER READINGS[8]	
		Actual Range	Proposed Range
Light	46	35–67	40–64
Medium	45	56–84	65–85
Heavy	47	85–>100	>85

[8] Meter readings are expressed as the absorbance difference between 800 and 930mμ. A value of less than 40 would indicate no smut.

[7] Absorbance difference = log of energy at 800 mμ − log of energy at 930 mμ.

ings varied from 35 to > 100. A low value denotes little or no smut in the sample, and conversely, a high value indicates a high degree of smut contamination.

A study was also made on 116 samples of nonsmutty wheat to determine what possible influence differences in moisture content and weight per bushel (kernel size) might have on meter readings. Since damaged kernels and foreign material may also affect meter readings, they were removed from the samples by hand separation. Moisture and weight-per-bushel values were obtained from the 116 official grade certificates representing samples of smut-free white wheat obtained from the Pacific Northwest area.

The 116 samples of nonsmutty white wheat were divided into three groups according to their test weight values (Table II). Tests made on samples of like test weight showed no significance at the 5% level for all three groups when meter readings were correlated with moisture contents.

TABLE II

RELATION BETWEEN MOISTURE CONTENT AND METER READINGS OF NONSMUTTY WHEAT

TEST WEIGHT	NO. OF SAMPLES	MOISTURE	METER READING	CORRELATION COEFFICIENT
lb		%		
58.0–60.0	44	8.4–15.4	21–46	−0.051
60.1–61.0	36	8.3–13.5	22–44	−0.218
61.1–63.8	36	8.2–12.6	20–49	−0.320

The relation was also determined between the 116 meter readings and respective test weight values. A correlation coefficient of −0.131 indicated no significance at the 5% level.

Therefore, neither moisture content nor test weight of the samples of wheat tested had a significant influence on the meter readings.

Evaluation of Methods for Determining Smut Content in Wheat

For the purpose of selecting the most practical method for determining smut content in wheat, each method was evaluated on the basis of eight important factors and the comparisons are given in Table III.

Discussion

The reference microscopic method served as a basis for calibrating and comparing the five proposed methods for determining smut content in wheat. The reference method is not practical for routine use

TABLE III

EVALUATION OF METHODS FOR DETERMINING SMUT CONTENT IN WHEAT

Factor	Methods						
					Light Reflectance		
	Basic Microscopic [a]	Light Transmittance	Sedimentation	Catalase Activity	Visual Comparison	Instrument	Light Absorption
Simplicity	Complex	Simple	Simple	Complex	Simple	Simple	Simple
Practicability	Poor	Good	Very good	Poor	Good	Good	Excellent
Rapidity [b] (minutes)	30	5	5	20	10	10	0.75
Accuracy	Good	Good	Good	Fair	Fair	Good	Good
Precision	Good	Good	Good	Fair	Fair	Good	Good
Correlation coefficient		+0.81**	+0.82**	+0.75**	[c]	−0.80**	+0.85**
Standard error (million spores)		0.21	0.71	3.5		2.3	0.26
Estimated cost of equipment	$500	$300	$20	$100	$50	$300	$500

[a] "Reference" method against which the five new methods were compared.
[b] Time per determination when run sequentially.
[c] 83.3% of these samples fell within the range of the standard with which it was matched.

because it is slow and tedious, but the other methods which were developed are more rapid and simple.

In the light-transmittance method, agar in the wash water is essential to keep the smut spores in suspension so that reliable meter readings and reproducible results between operators can be obtained. It is believed that the colorimeter responds to the number of heat-resistant thick-walled teliospores of smut present in the suspension, since most other influences have been physically or chemically eliminated. This rapid light-transmittance method for determining smut content in wheat is simple, accurate, and practical. After brief training, nontechnical personnel can make accurate determinations. Fifteen minutes are required for a single determination. When the samples are run sequentially each sample requires only about 5 minutes as compared to about 30 minutes when the basic microscopic method is used. However, the method has a serious limitation, in that the smut spores must be removed from the wheat kernels by the time-consuming washing procedure. It would be more desirable to test bulk wheat directly for smut content, since time is a prime factor in grain inspection work.

The sedimentation test for quantitative determination of smut content in wheat is simple, accurate, inexpensive, and rapid. The most important feature in this quick test is its economy. It is estimated that it would cost an inspection laboratory already equipped with a balance, about $20 for sufficient equipment to determine the smut content on 100 samples of wheat daily. The daily cost for reagents is nominal and each sample requires only about 5 minutes per determination. This test could be used in areas of low smut incidence where an occasional test for smut may be required. This technique was found to be helpful in differentiating between smut and other forms of mold infection in samples of questionable smutty wheat.

The catalase activity method requires inexpensive equipment and reagents and gives fairly reproducible results, but has the disadvantage that it takes approximately 20 minutes per determination when samples are run sequentially. Also, catalase activity decreases during the reaction even at low temperatures because of its partial destruction. In addition, the accuracy of this method may suffer from side reactions between the strong oxidizing action of permanganate and impurities. However, this phenomenon was not observed in filtered samples. Another disadvantage stems from the fact that working at 10°C. is less convenient than working at room temperature. However, with the sacrifice of some degree of accuracy this indirect procedure for determining smut content could be performed at room temperature.

Needless to say, this chemical method would be very impractical in inspection offices where several hundred samples must be graded within a day's time.

The visual comparison test is rapid (10 minutes) and simple to perform; filter paper standards are easily prepared, and the cost per determination is negligible. The Agtron reflectance test, however, could be performed almost as rapidly and with better accuracy than the visual comparison of smut deposits, because it is an objective method.

A number of points should be considered in using the light-absorption method. It has distinct advantages over the other four methods because of its practicability, simplicity, rapidity, accuracy, and good reproducibility. In addition the light-absorption method eliminates the subjective influences usually found in visual grading. The instrument is commercially available at a cost of approximately $500. This procedure should find practical application in grain inspection work where rapid analysis is desirable. With this instrument a single operator can test 500 samples of wheat for smut content in an 8-hour working day.

In all of the proposed methods, dockage was removed from the wheat before the analysis proceeded. Dockage includes such macro contaminants as large stems, weed seeds, and dirt particles, which are removed by passing over a riddle through a series of screens and by means of air. Even so, it is obvious that micro contaminants (non-smut particles) are present in smut-water suspensions, decreasing the accuracy for determining smut concentration in dockage-free wheat. However, in the 138 samples of wheat analyzed for smut content in this study, this apparent factor of contamination did not significantly alter the relation between method results and spore counts, as evidenced by the high correlations obtained in all five proposed methods.

Since the presence of smut within kernels of wheat that were presumedly smut-free has been established by Griffith *et al.* (4) and later by Zscheile (9,10), the light-absorption method, which determines the external as well as the internal infestation of smut, should be more accurate for measuring total smut contamination directly in samples of bulk wheat.

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Literature Cited

1. Birth, G. S. Looking inside fruit. Agr. Marketing **2:** 2–3 (1957).
2. Birth, G. S., Norris, K. H., and Yeatman, J. N. Nondestructive measurement of internal color of tomatoes by spectral transmission. Food Technol. **11:** 552–557 (1957).
3. Cherewick, W. J. An improved method of determining the smut spore load on cereal seed. Can. J. Research **C22:** 120–126 (1944).
4. Griffith, R. B., Zscheile, F. P., and Oswald, J. W. The influence of certain environmental factors on expression of resistance to bunt in wheat. Phytopathology **45:** 428–434 (1955).
5. Heald, F. D. The relation of spore load to the percent of stinking smut appearing in the crop. Phytopathology **11:** 269–278 (1921).
6. Morgulis, S., Beber, M., and Rabkin, I. Studies on the effect of temperature on the catalase reaction. I. Effect of different hydrogen peroxide concentrations. J. Biol. Chem. **68:** 521–563 (1926).
7. Norris, K. H. Measuring light transmittance properties of agricultural commodities. Agr. Eng. **39:** 640–643, 651 (1958).
8. Von Euler, H., and Josephson, K. Über Katalase. I. J. Justus Liebigs Annalen der Chemie **452** (2): 158–181 (1927).
9. Zscheile, F. P. Search for chlamydospores in wheat seeds grown under conditions favorable to bunt expression. Phytopathology **46:** 182–183 (1956).
10. Zscheile, F. P., and Anken, M. Limited development of Tilletia chlamydospores within wheat kernels. Phytopathology **46:** 183–186 (1956).

ON THE ABSORPTION OF LIQUID WATER BY THE WHEAT KERNEL[1]

H. A. BECKER

ABSTRACT

The absorption of liquid water by the wheat kernel proceeds by a heterogeneous mechanism. There is a very rapid initial absorption in which the pericarp is saturated by capillary imbibition. The average moisture gain due to this absorption is 0.045 g. per g., dry basis, at 22°C., but decreases with increasing temperature. Subsequent absorption is directly proportional to the square root of the period of immersion and is described by the diffusion equation

$$1 - \overline{M} = \frac{2}{\sqrt{\pi}} \frac{S}{V} \sqrt{Dt}$$

where $1 - \overline{M}$ is the relative moisture gain and S/V is the surface-to-volume ratio. The average diffusion coefficient is given by the Arrhenius relation

$$D = 30 \exp.(-12300/RT)$$

The activation energy, 12.3 kcal. per mole, is identical with that previously found for vacuum drying, but the diffusion coefficient itself is only one-fifth to one-third as large. The effective moisture content at the surface of an immersed wheat kernel is 0.75 g. per g. and is identical with the critical moisture content in drying.

Previous studies (3,4) dealt with the drying of wheat in a moisture-free atmosphere and were designed to elucidate the process of water diffusion in the wheat kernel under desorption conditions. The validity of these results under adsorption conditions is conjectural, however, for the water diffusion coefficients of polymers are often sensitive to the direction of changes in concentration. The present investigation was undertaken to obtain data on the absorption of water by wheat kernels fully immersed in liquid water. The present and previous studies describe the nature of water diffusion in the wheat kernel under opposite extremes of ambient conditions and thus define the limits of the behavior to be expected between them. The present study also provides information which is basic to the commercially important practice of tempering wheat.

Materials and Methods

Wheat. The Thatcher wheat was from the same lot used in a previous study (3) of the vacuum drying of wheat. Samples were prepared

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with initial moisture contents[2] ranging from 7.1 to 50%, dry basis. The conditions of tempering and storage are described in the above-mentioned paper.

Methods. Samples for moisture determination were ground at the "medium" setting in a Hobart coffee mill; this produced a product which was granular rather than powdery. The time the sample remained within the mill appeared to be well under 1 second, and the product was received directly in the bottles used for further storage. In general, every precaution was taken to minimize moisture losses during handling of the samples destined for moisture assay.[3]

Moisture contents were determined by drying the ground samples for a period of 4 hours at 110°C. in an oven evacuated by an efficient vacuum pump. This method of moisture determination had previously been found to give excellent results (3).

The absorption of water by wheat was studied as a function of time, temperature, and initial moisture content. Samples were placed in wire gauze baskets and immersed in a stirred water bath controlled within 0.1°C. of the set temperature. At the end of the absorption period the samples were quickly removed and superficially dried by manual rolling of the kernels on a large filter paper until they lost the glistening appearance associated with the presence of a surface film of water. The filter paper was then quickly rolled into cylindrical shape, the wheat inside, and the wheat was thence transferred to a storage bottle in a total time (after cessation of drying) of roughly 5 seconds. Despite the speed of handling, it is still possible that significant losses of moisture occurred after surface drying at the higher temperatures because of evaporation.[4] The effect of such losses will be dis-

[2] All moisture contents in this paper are expressed in g. per g., dry basis.

[3] It has been pointed out by a reviewer that the USDA prescribes a two-stage moisture method for cases where the initial moisture content is higher than 15% moisture content, dry basis (or 13%, wet basis): namely, that wheat of high moisture content be equilibrated at ambient conditions to grinding. This is, in general, a sound criticism and will be heeded in future studies. Nevertheless, it is thought that moisture losses in grinding were not serious in the present case for the following reasons: 1) samples were reduced to a granular rather than a pulverized (floury) state so that, from the point of view of diffusion, residence times within the mill appear to have been safely short; 2) the evaporation of moisture within the mill would establish an equilibrium atmosphere (assuming negligible through-circulation of air), and the amount of vaporizable moisture would therefore be severely limited by the dearth of available gas volume; and 3) the samples ground were small (10 g.) so that heating of the grinding chamber was negligible, as ascertained by tactile examination of the grinding plates. It should be noted, in the latter connection, that fine grinding of dry samples produces vastly more heat than coarse grinding of rather moist samples.

An estimate of the maximum moisture loss possible during grinding is easily made, using the approximate diffusion equation $(\bar{m} - m_s)/(m_o - m_s) = (2/\sqrt{\pi})\ (S/V)\ (\sqrt{Dt})$. Assume, for the sake of argument, that the ground wheat particles are spherical, that the temperature is 30°C., and that the atmosphere surrounding a particle is perfectly dry (corresponding to high vacuum or to a high rate of ventilation with dry air). The diffusion coefficient is then 10^{-7} cm.^{2} per second (3), the surface moisture content is $m_s = 0.10$ g. per g. (3), and for the ratio S/V one can substitute $S/V = 6/D_v$, where D_v is the diameter of the particle. With these substitutions, the diffusion equation can be written $(\bar{m} - m_o)/(m_o - m_s) = 2.14 \times 10^{-3}\sqrt{t}/D_v$. Then, in 1 second the reduction in free moisture content (moisture above 0.10 g. per g.) is 2.14% for a particle 1 mm. in diameter, and 21.4% for a particle 0.1 mm. in diameter. The main weight fraction of ground wheat in the present study was in the half-millimeter range. At a moisture content of 0.30 g. per g. such a particle would lose 0.0006 g. per g. moisture in 1 second, and the loss would increase in proportion to the square root of the time.

[4] The maximum possible magnitude of the moisture loss after the completion of surface drying is

cussed at an appropriate point further on in the paper.

In a first series of experiments, the moisture gain during the absorption period was calculated from the initial and final moisture contents, both of which were determined by the vacuum-oven method. In the second series only the initial moisture content was determined, the size of the sample was reduced from 10 g. to 2 g., and the moisture gain was calculated from the gain in weight of the sample. Distinction between the two series is made because the absorption-time relations were slightly different. The second method of determining moisture gain is superior because it reduces handling of grain at high moistures. It should be noted that in series 1 the surface-moist wheat kernels obtained from the absorption experiments were allowed to equilibrate in sealed bottles for at least 3 to 4 hours prior to grinding.

Experiments were carried out at temperatures of 0° to 70°C., and absorption periods ranged from several minutes to 8 hours.

Mathematical analysis (3) of nonstationary-state diffusion in solids of arbitrary shape shows that at times in the neighborhood of zero and under the conditions

$$m = m_0 \text{ at } t = 0$$

$$m = m_s \text{ at the bounding surface at } t > 0$$

$$D = \text{constant}$$

the integral diffusion equations are of the general form

$$\overline{M} = 1 - \frac{2}{\sqrt{\pi}} X + BX^2 \qquad (1)$$

where

$$M \equiv \frac{\overline{m}_s - \overline{m}}{\overline{m}_s - m_0}$$

$$X \equiv \frac{S}{V}\sqrt{Dt}$$

and

m_0 = initial, uniform moisture content, dry basis, ... g/g

$\overline{m}$ = average moisture content at given absorption time, dry basis, ... g/g

m_s = effective moisture content at the bounding surface at times greater than zero, dry basis, ... g/g

limited by the rate at which heat can be supplied to the zone of evaporation. Consider a sample of wheat at 50° C. with an average moisture content of 0.30 g. per g. Heat for evaporation will come solely from the sensible heat of the kernel so long as the temperature in the zone of evaporation remains above the ambient temperature. The sensible heat above an ambient temperature of 25° C. is 1.3 (0.6) (25) = 20 cal. This could evaporate a maximum of 0.036 g. per g. of moisture. However, moisture losses in the present experiments were probably considerably lower than this because 1) some of the heat was lost during the process of surface drying on the filter paper; and 2) in the short time available, only a fraction of the remaining heat could have been used for evaporation since the grain received in the storage bottles was still very warm.

t = absorption time ..... sec
S = area of bounding surface ..... cm^2
V = volume (e.g., of wheat kernel) ..... cm^3
D = diffusion coefficient ..... cm^2/sec
B = dimensionless constant

At small values of X this equation approximates to

$$1 - \overline{M} = \frac{2}{\sqrt{\pi}} X \tag{2}$$

or, in terms of the experimental variables,

$$\overline{m} - m_o = k_o \sqrt{t} \tag{3}$$

where

$$k_o \equiv \frac{2}{\sqrt{\pi}} (m_s - m_o) \left(\frac{S}{V} \right) \sqrt{D}.$$

Therefore, at small values of X, the moisture gain of a wheat sample immersed in water should be approximately proportional to the square root of the absorption time, if the diffusion equation is applicable. Figure 1, which shows typical data from series 2, shows that such a relation is indeed obeyed. However, the curves do not extrapolate to

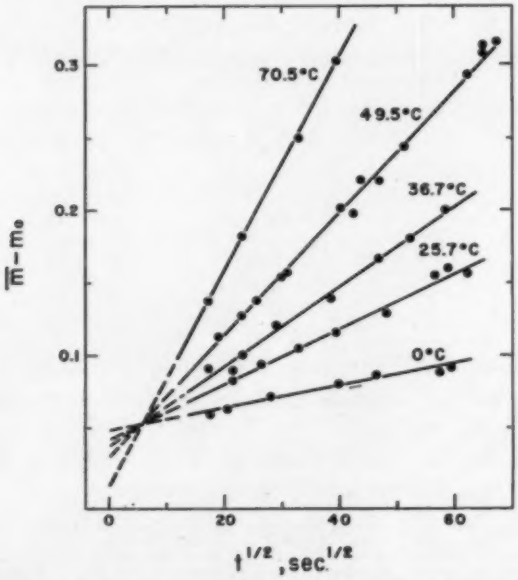

Fig. 1. Data from series 2 for an initial moisture content of 0.25 g. per g., showing the linear relation between the moisture gain and the square root of the absorption time.

zero moisture gain at time zero, but to an intercept $\Delta\overline{m}_i$. The experimental data were therefore analyzed statistically to obtain the slopes, k_o, and the intercepts, $\Delta\overline{m}_i$, of the linear regression of the moisture gain on the square root of the absorption time.

The Initial Moisture Gain, $\Delta\overline{m}_i$. If the linear relation between the moisture gain and the square root of the absorption time indeed signifies obedience to Fick's Law with a constant diffusion coefficient, then the occurrence of the nonzero intercept $\Delta\overline{m}_i$ means that there is a very rapid initial absorption of water which is due to another phenomenon than slow diffusion into the interior of the kernel. The nature of this phenomenon is evident from the structure of the wheat kernel, for the outermost layer, the pericarp, is highly porous and should quickly become saturated by capillary imbibition. (Jones (10) appears to have been the first to distinguish this aspect of liquid water absorption by wheat.) It therefore appears that the intercept $\Delta\overline{m}_i$ is a measure of the amount of water required for saturation of the pericarp. The pericarp constitutes approximately 4.8% of the dry weight of the wheat kernel (15), and so its moisture content at saturation is (dry basis)

$$m_p = m_o + \Delta\overline{m}_i / (0.048), \qquad (4),$$

assuming that the initial moisture distribution is uniform. Figure 2 shows that saturation moistures so calculated from the experimental data were in the range 1.0 to 1.2 g. per g. at room temperature and decreased markedly with increasing temperature. The values appear to be independent of initial moisture content at levels above 0.14 g.

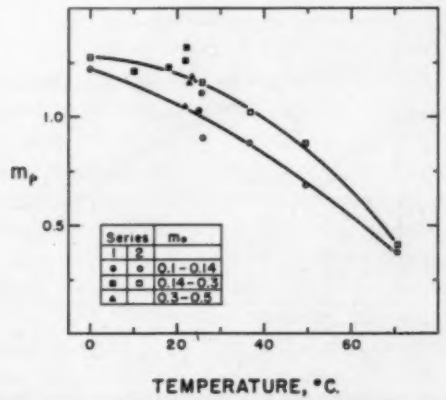

Fig. 2. The saturation moisture of the pericarp as a function of temperature and initial moisture content.

per g. but are significantly lower at moistures of 0.11 and 0.14 g. per g. One would expect the saturation moistures to be practically independent of the initial moisture level, but the reason for their strong dependence on temperature is not wholly clear. While evaporative moisture losses during handling of the grain after surface drying on filter paper may have been partly responsible, it seems likely that the effect is largely due to drainage of the grosser capillary structure of the pericarp by the filter paper itself, this process being promoted by the lowering of the surface tension of water with increasing temperature. The presence of air bubbles (whose volume would increase with rising temperature) in the pericarp may also have been a contributing factor.

The presence of unexpelled air was illustrated by a number of experiments in which wheat with an initial moisture content of 0.147 g. per g. was soaked in water at the point of incipient boiling (under vacuum) at 25°C. It was observed that bubbles were liberated at the surfaces of the kernels in the first 2 minutes of exposure. The value found for the saturation moisture of the pericarp in these experiments was 1.60 g. per g., an increase of 0.5 g. per g. over the value of atmospheric pressure. The slope, k_o, of the linear relation between the moisture gain and the square root of the absorption time, however, was unaffected by this variation in total pressure, in further proof that this relation indeed reflects a diffusion-controlled mechanism.

Figure 3 shows that the initial moisture gains $\Delta\overline{m}_i$ in the rather narrow range of initial moistures, 0.1 to 0.25 g. per g., of ordinary practical interest were not significantly dependent on the initial moisture

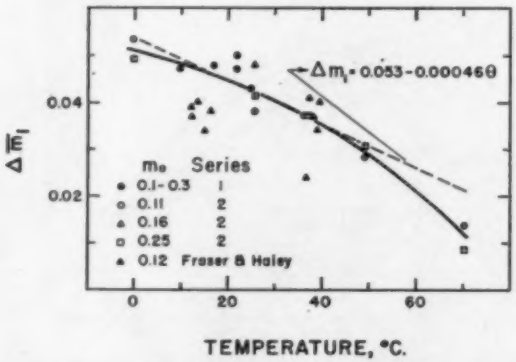

Fig. 3. The initial moisture gain as a function of temperature and initial moisture content for the practically important range of initial moisture 0.10 to 0.25 g. per g.

level. The data for temperatures in the range 10° to 50°C. are represented with fair accuracy by the relation

$$\overline{\Delta m_i} = 0.053 - 0.00046\ \theta \qquad (5)$$

where θ is the temperature in °C. Also shown are values of $\overline{\Delta m_i}$ calculated from the early data of Fraser and Haley (7). These are in good agreement with the present results at 40°C. but show no trend with temperature and so are somewhat lower at 15°C.

In general, it is evident that the experimental value of the initial moisture gain is more or less dependent on the treatment of the wheat kernels following emersion from the water bath. Removal of the adhering film of liquid water by rolling the kernels on a filter paper, as in the present study, gives results of high precision. However, it is desirable that $\overline{\Delta m_i}$ should, in some future study, be determined under conditions simulating more closely those prevailing in commercial tempering.

Although the rapidity of saturation of the pericarp is clearly ascribable to capillary imbibition, it does not follow that the water imbibed ($\overline{\Delta m_i}$ or m_p) is held solely by weak capillary forces. It can hardly be doubted that a part of it swiftly diffuses into the thin cell walls (the substance of the pericarp) and is held there by adsorptive forces. Hence m_p consists of two fractions: a capillary-held, free-water fraction, and an adsorbed, bound-water fraction. The two are probably of comparable magnitude, the critical moisture content of cellulose (the moisture content above which a surface film of free water appears) being approximately 0.3 g. per g. The capillary-held fraction appears to be the one which was strongly dependent on temperature in the present study.

Relating to the section which follows, the critical moisture content of the pericarp material appears to be considerably lower than that with respect to the entire kernel, which will be shown to be approximately 0.75 g. per g.

The Effective Surface Moisture Content, m_s. Equations 2 and 3 show that if the diffusion coefficient is independent of moisture content in the range studied, the product of the slope k_o and the kernel volume-to-surface ratio V/S should be a linear function of the initial moisture content and should extrapolate to an intercept $m_o = m_s$ at $k_oV/S = 0$, where m_s is the effective moisture content at the bounding surface. Figure 4 shows that the data fulfill this expectation reasonably well and indicates that the effective moisture content at the surface of a wheat kernel immersed in liquid water is approximately 0.75 g. per g. The volume-to-surface ratio was in each case evaluated at the initial

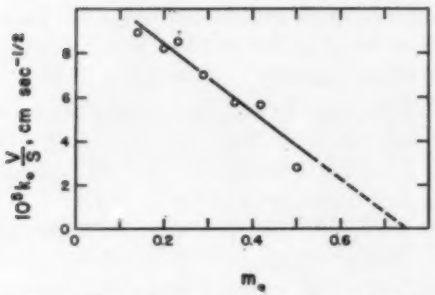

Fig. 4. The product k_oV/S as a function of the initial moisture content at 22°C., showing the regression on an effective surface moisture content of 0.75 g. per g.

moisture content,[5] using the formula previously given (formula 5): $V/S = 0.91\ r_v/3$, where r_v is the radius of a sphere with volume equal to that of the wheat kernel. The volume-to-surface ratio at a moisture content of 0.10 g. per g. was 0.0515 cm. and increased with increasing moisture content in proportion to $(0.69 + m_o)^{1/3}$, where 0.69 cm.^{3} per g. is the specific volume of moisture-free wheat (3).

The moisture gain at 22°C. and at a constant absorption time of 15 minutes was determined at a large number of initial moisture contents and is shown as a function thereof in Fig. 5. As might be expected, the relation is very similar to that in Fig. 4, and the intercept is again approximately $m_o = m_s = 0.75$ g. per g.

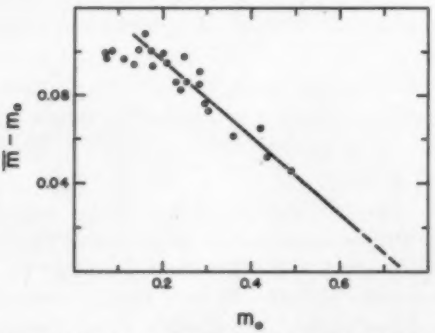

Fig. 5. The moisture gain in an absorption time of 15 minutes as a function of initial moisture content at 22°C., showing the regression on an effective surface moisture content of 0.75 g. per g.

[5] In vacuum drying (3) V/S was evaluated at a moisture content of 0.10 g. per g. The present work, however, embraced a much wider range of initial moistures, and consequently did not permit treatment of V/S as a constant. The volume-to-surface ratio was therefore based on the initial moisture content, in order to account at least partially for the effects of appreciable variation of kernel volume with the moisture content.

The effective surface moisture content with respect to diffusion when wheat is immersed in *liquid* water appears to be practically identical with the critical moisture content in drying (i.e., the moisture content above which drying is controlled by evaporation from the free-water surface which then envelops the kernel). Simmonds *et al.* (16) found the critical moisture content of wheat to be in the range 0.7 to 0.8 g. per g., and 0.75 is an acceptable average value of their results. This identity with the critical moisture content is plausible theoretically and attaches a concrete significance to the surface moisture level prevailing under conditions of *liquid* water absorption.

The Diffusion Coefficient, D. The diffusion coefficient D is given directly by equations 2 and 3 once the effective surface moisture content is known. Figure 6 shows diffusion coefficients calculated from the

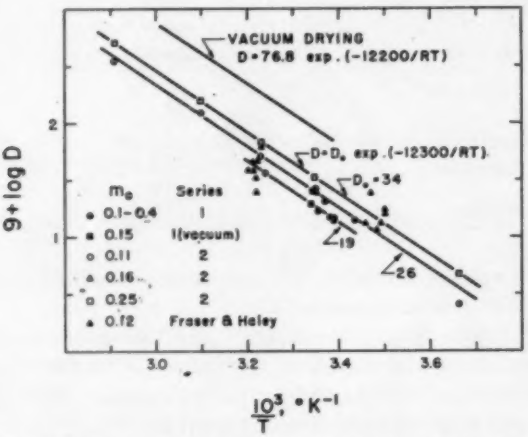

Fig. 6. The diffusion coefficient as a function of the reciprocal of the absolute temperature, showing obedience to Arrhenius's relation.

present data and those of Fraser and Haley (7), as a function of the reciprocal of the absolute temperature, $°K^{-1}$. The data for series 2, which covered a wide range of temperatures, give for the energy of activation in the Arrhenius relation

$$D = D_0 e^{-E/RT} \qquad (6)$$

the value E = 12.3 kcal. per mole. This is in close agreement with the value E = 12.2 kcal. per mole previously found in vacuum-drying experiments (3) in which the initial moistures ranged from 0.13 to 0.25 g. per g. It therefore appears that the activation energy of the diffusion process is independent of the direction of changes in moisture level.

However, the coefficient for the diffusive part of liquid water absorption is appreciably smaller than the coefficient for vacuum drying; the value of D_o in the Arrhenius relation for an energy of activation of *12.2* kcal. per mole is 16 to 29 cm.^{2} per second, compared with 76.8 cm.^{2} per second in vacuum drying. (It is emphasized that this comparison is based on an energy of activation of *12.2* kcal. per mole, not *12.3*, and that the values quoted for D_o are for this reason different from those in Fig. 6.)

The diffusion coefficients calculated from Fraser's and Haley's data (7)[6] fall within the range enclosed by series 1 and 2 (Fig. 6 and Table I), but the energies of activation are considerably lower (Table I), being in the range 5 to 9 kcal. per mole.

TABLE I

DIFFUSION OF WATER INTO THE WHEAT KERNEL

PRESENT DATA	10^6 D_m*	E
	cm^2/sec	*kcal/mole*
Thatcher, series 1	2.4–3.1	12.3
series 2	1.8	
Fraser and Haley		
Marquis	2.1	7.4
Turkey	3.3	5.1
Baart	2.1	2.3
Hard Federation	2.8	7.6
Club	1.9	8.7
Red Russian	1.8	4.9

Practical Applications of the Data. Figure 7 shows a general relation of all the data for temperatures in the range 10° to 50°C. and initial moistures in the range 0.10 to 0.30 g. per g., *dry basis*. The parameters were calculated from the experimental relations

$$1 - \overline{M} = \frac{\overline{m} - m_o - \Delta\overline{m}_i}{m_e - m_o}$$

$$D = 30 \; exp\,(-12300/RT) \qquad (6')$$

$$m_e = 0.75$$

$$\Delta\overline{m}_i = 0.053 - 0.00046\,\theta \qquad (5)$$

These relations, combined with equation 2, can also be used for practical prediction of the moisture gain as a function of the independent variables. Figure 7 therefore gives an indication of the accuracy with which such predictions can be made. It is seen that the only data which

[6] Fraser and Haley gave no data on kernel size or weight; so a value of V/S of 0.052 cm. was assumed.

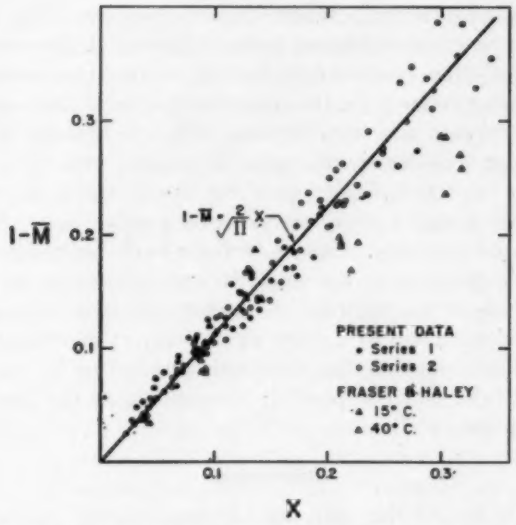

Fig. 7. Generalized correlation of the data, using empirical relations for the diffusion coefficient and the initial moisture gain.

deviate remarkably from equation 2 with the above values of D, m_e and $\Delta\overline{m}_i$ are those of Fraser and Haley (7) for 40°C., which are approximately 20% low in the value of $1 - \overline{M}$. However, since their data for 15°C. are in excellent agreement with the present results, it is not unlikely that those for 40°C. are in error, owing to variation of grain temperature during the absorption period, to loss of moisture in handling, or to other causes. Such effects would almost certainly be more pronounced at the higher temperature. Moreover, the fact that the energies of activation for diffusion calculated from their data are so much lower than the present value and are in such poor agreement among themselves (Table I), makes the data for 40°C. doubly doubtful. The effects of variety, protein content, and other relevant constitutional factors on the absorption of liquid water by wheat should therefore be reinvestigated before any firm conclusions about their influence are made. It is noteworthy that these factors have no apparent effect on the drying of wheat (3).

It appears from Fig. 7 that the differences in the calculated diffusion coefficient between series 1 and 2 are more artificial than real. The data of series 1 generally gave the higher values of the initial moisture gain $\Delta\overline{m}_i$ (Fig. 3), but the lower values of the slope k_o (and, hence, of the diffusion coefficient, Fig. 6). Cancellation of these opposing effects pro-

duced the good agreement between the two series seen in Fig. 7; in fact, there was no practical difference between the total moisture gains at given values of time, temperature, and initial moisture content. However, the data of series 2 are the more reliable as to the values of the initial moisture gain and the diffusion coefficient because of the superior method used to determine the total moisture gain.

It should be noted, finally, that the applicability of equation 2 over the entire range of experimentation is partly due to the *a priori* assumption of its validity, made to define a basis for treatment of the data. Therefore, because the analysis was prejudiced in its favor, equation 2 should be regarded as an imperfect, though highly expedient, approximation to a more nearly correct general solution. A subtler approach, such as that previously applied to the vacuum drying of wheat (3), was not possible here, owing to the heterogeneity of the absorption mechanism.

Discussion

Hinton (9) found that removal of the testa, the botanical layer directly beneath the pericarp or outermost bran layer, approximately tripled the rate of liquid water absorption from a capillary tube, 0.35 mm. inside diameter, held to the surface of a wheat kernel. His work corrected an earlier notion that the hyaline layer, which lies directly beneath the testa, is a layer of exceptionally low water permeability (8, 10). In consequence of Hinton's and of the earlier work, it has often been suggested that the resistance to water penetration into the wheat kernel resides chiefly in one or the other of these outer layers, and it has even been generalized that the hyaline layer should control the drying of wheat (13). However, these theories disregard the possibility of changes in mechanism when the outer layers are removed, and the following, alternative explanation seems to give a more forceful interpretation of the facts.

The basic process by which gradients in the chemical potential of moisture in wheat are removed, in liquid water absorption as in drying, is diffusion. However, in liquid water absorption, the gross process of diffusion may be materially accelerated by the agency of natural capillaries and of structural flaws and cracks. It appears that these channels can facilitate the conduction of liquid water into the interior of the wheat kernel. From them it diffuses into the substance of the kernel, and its flow in them is maintained by the draining action of this diffusion. The rate of liquid water absorption is therefore controlled by the rate at which water can diffuse into the substance of the kernel, and the energy of activation of this process should be practical-

ly the same as in drying (as actually found in the present study). However, the gross value of the diffusion coefficient is increased, for the appearance of sources of liquid water within the kernel is equivalent to subdivision of the kernel into shapes of smaller size and greater total surface area.

The testa appears to be impervious only in the sense that it lacks an appropriate capillary structure and so seals the capillary channels by which liquid water might be conducted into the interior of the kernel. The only part of the kernel not so sheathed is the germ end, and the rate of absorption is abnormally high in this region (8,17); but, because the germ area is small, the effect of this on the total rate of absorption cannot be large.

The effect of the testa, it thus appears, is to prevent direct access of liquid water to the interior of the kernel. Presumably, any water then entering does so by diffusion alone, except, of course, in the germ area. There is no reason, be it noted, for assuming that the testa importantly limits a process of pure diffusion. The water diffusion coefficient for the testa may well be lower than that for the interior of the kernel, but it would have to be many orders of magnitude lower if this thin layer were to constitute a controlling resistance.

If the foregoing reasoning is correct, the diffusion coefficients based on total kernel size should be nearly normal and directly comparable with those determined from drying experiments. Indeed, the problem is to explain why the calculated diffusion coefficients for liquid water absorption are only one-fifth to one-third as large as those previously found for vacuum drying, and not why they are larger. It is unlikely that there is any difference in the process of diffusion under these different conditions, for the identity of the energies of activation (12.3 and 12.2 kcal. per mole for water absorption and for drying, respectively) indicates that the mechanism is the same. Thermodynamic factors should therefore be chiefly responsible; it is generally held that the motivating force in diffusion is the chemical potential and that the Fickian diffusion coefficient can therefore be expressed as (12,14)

$$D = ukT\frac{\partial \ln f}{\partial \ln c} \qquad (7)$$

where u, f, and c are, respectively, the mobility, relative vapor pressure, and concentration of the diffusing species, and k is Boltzmann's constant. Because of the very appreciable hysteresis in the adsorption-desorption equilibria of water with wheat or its constituents (1,6), the factor $\partial \ln f/\partial \ln c$ is considerably larger in desorption than in adsorption, at least in the range of moistures presently concerned. This, then,

explains a part, and perhaps nearly all, of the observed difference in the diffusion coefficients. A small part (at most, 20%) may be due to the inexactness of the mathematical method used to evaluate the diffusion coefficient in the present study. Consideration of the entropy of activation as a responsible agency appears to be irrelevant here, for changes in the entropy of activation for diffusion in polymers are quite generally accompanied by proportional changes in the energy of activation (2), and such a change did not occur.

The foregoing is, of course, not the only possible explanation of the observed phenomena, but it appears to the author to be the simplest one and, indeed, the only one that accounts for all the salient features of the absorption-time-temperature relation without encountering serious, invalidating contradictions.

It has often been suggested that the penetration of water into the endosperm involves a capillary mechanism, and, in favor of this, the fact has been quoted that the wheat kernel indeed possesses an appropriate capillary structure. However, the mere existence of a capillary structure does not mean that the *rate* must be capillary-controlled. The basic conclusion of the present investigation in this regard, that the rate of penetration is diffusion-controlled, has no plausible alternative; for the capillary mechanism, compounded of a surface-tension driving force and a viscous resistance, is inflexible as to temperature dependency and cannot possibly explain an energy of activation of 12.3 kcal. per mole.

The possible role of the capillary system is thus narrowed to the potentially important one suggested earlier; i.e., the capillaries may conduct liquid water into the interior of the wheat kernel and so accelerate the process of diffusion into the *substance* of the kernel by effectively increasing the exposed surface area. Although this possibility has been discounted in the preceding argument, the question is by no means closed.

An important obstacle to capillary flow remains to be mentioned: If the capillaries are initially filled with air, they will tend to become vapor-bound when liquid water enters their termini. The present work has shown that this effect is important even in the relatively exposed pericarp. On the other hand, if cracking of the kernel takes place before an advancing front of moisture, such cracks may well fill with water and may then serve to accelerate the process of diffusion.

As to the role of the testa, Hinton (9) has expressed a viewpoint similar to that advanced here: In the concluding sentences of his highly relevant paper he states, "Thus, though the rate of movement is three to six times faster through the [bare] endosperm than through

the testa, the distances involved in the endosperm make it of prime importance in establishing the time required for complete conditioning of wheat. The practical significance of the resistance of the testa is that it limits the water taken up by wheat during washing to that which can be absorbed by the pericarp."

It may be concluded that while the hypotheses underlying the present interpretation of water absorption by the wheat kernel are certainly oversimplified with regard to the exact state of affairs inside the kernel, they are fruitful in yielding the mathematical form of the law governing absorption and bring to the surface new facts which must be considered in any more detailed investigation.

Literature Cited

1. Babbit, J. D. Observations on the absorption of water vapour by wheat. Can. J. Research **27F:** 55–72 (1949).
2. Barrer, R. M. Permeability in relation to viscosity and structure of rubber. Trans. Faraday Soc. **38:** 322–331 (1942).
3. Becker, H. A. A study of diffusion in solids of arbitrary shape, with application to the drying of the wheat kernel. J. Appl. Polymer Sci. **1:** 212–226 (1959).
4. Becker, H. A., and Sallans, H. R. A study of internal moisture movement in the drying of the wheat kernel. Cereal Chem. **32:** 212–226 (1955).
5. Becker, H. A., and Sallans, H. R. A study of the desportion isotherms of wheat at 25°C. and 50°C. Cereal Chem. **33:** 79–91 (1956).
6. Bushuk, W., and Winkler, C. A. Sorption of water vapor on wheat flour, starch, and gluten. Cereal Chem. **34:** 73–86 (1957).
7. Fraser, C. W., and Haley, W. L. Factors that influence the rate of absorption of water by wheat. Cereal Chem. **9:** 45–49 (1932).
8. Haltmeier, O. Wo und wie dringt das Wasser in das Getreidekorn ein? Muehle **70:** 1165 (1933).
9. Hinton, J. J. C. Resistance of the testa to entry of water into the wheat kernel. Cereal Chem. **32:** 296–306 (1955).
10. Jones, C. R. Observations on the rate of penetration of water into the wheat grain. Milling **113:** 80–86 (1949).
11. Jones, C. R. Recent British studies of wheat conditioning and drying. 3rd Internationaler Brotkongress, Hamburg (1955).
12. Jost, W. Diffusion in solids, liquids, gases. Academic Press, Inc.: New York (1952).
13. McEwen, E., Simmonds, W. H. C., and Ward, G. T. The drying of wheat grain. Part III. Interpretation in terms of its biological structure. Trans. Inst. Chem. Engrs. (London) **32:** 115–120 (1954).
14. Onsager, L., and Fuoss, R. M. Irreversible processes in electrolytes. Diffusion, conductance, and viscous flow in arbitrary mixtures of strong electrolytes. J. Phys. Chem. **36:** 2689–2778 (1932).
15. Shetlar, M. R., Rankin, G. T., Lyman, J. F., and France, W. G. Investigation of the proximate chemical composition of the separate bran layers of wheat. Cereal Chem. **24:** 111–122 (1947).
16. Simmonds, W. H. C., Ward, G. T., and McEwen, E. The drying of wheat grain. Part I. The mechanism of drying. Trans. Inst. Chem. Engrs. (London) **31:** 265–278 (1953).
17. Swanson, C. O., and Pence, R. O. The penetration rate of water in wheat during tempering as disclosed by College Mill trials. Am. Miller **58:** 435–436 (1930).

CHROMATOGRAPHIC METHOD FOR FRACTIONATING GLOBULINS OF CORN[1]

T. A. McGuire, E. M. Craine,[2] and R. J. Dimler

ABSTRACT

The globulins in water extracts of corn were fractionated by ion-exchange chromatography on carboxymethylcellulose (CMC). To increase solubility of the proteins at low pH, phytate ions were removed before chromatography. Proteins were adsorbed on unbuffered columns of CMC at low pH and ionic strength (1% acetic acid) and were desorbed by stepwise increases in the ionic strength of the eluant at constant pH (2.8). Rechromatography of components established that a true fractionation was obtained. Under conditions used, the unadsorbed material contained a true protein fraction, along with most of the yellow pigments and the nucleic acids of the extract. Evidence for an active nuclease was found.

Fundamental studies on the proteins of corn in progress in this laboratory required methods for extraction, detection, and measurement of individual protein fractions. In the work of Craine and Fahrenholtz (3) the proteins in water extracts of corn were detected by electrophoresis but resolution often was not good enough to permit quantitative measurement. In preliminary investigations chromatography of the globulins on carboxymethylcellulose (CMC) gave better resolution. In addition the protein recovery was high. Chromatography therefore had promise for determining the globulins as well as for purifying and fractionating them.

The proteins studied were extracted from corn with water and designated as globulins for easy reference (3). When the extract mixture is purified, these proteins appear to have isoelectric points in the pH range from 3 to 5 and are completely soluble below pH 3. This solubility characteristic is masked in the crude extracts by formation of insoluble protein-phytate complexes in the acidic pH range. Because of this effect on protein solubility, removal of phytate ions (3) proved necessary as a preliminary to chromatographic fractionation of the proteins on CMC.

Materials and Methods

The corn meal used for preparations of protein was that described by Craine and Fahrenholtz (3). It was made from a commercial sample

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of Schwenk 13 corn[3] and contained 8.4% moisture, 1.72% nitrogen, and 0.30% phosphorus.

Nitrogen was determined by a conventional micro-Kjeldahl procedure using mercuric oxide as a catalyst in the digestion. Total phosphorus was determined by a modification of the method of Truog and Meyer (12).

Dowex-1 (10X) was cycled twice through the hydroxide and chloride forms. The resin was then converted to the acetate form by washing with a solution of sodium acetate until the eluate was free of chloride ions.

Hercules CMC-30 was purified by treatment with 2*N* hydrochloric acid for 1 hour, followed by washing with water (settling and decantation) to remove excess acid and fine particles of CMC. This cycle was repeated until further treatment with 2*N* HCl produced no coloration in the supernatant solution. Finally the CMC was washed with 95% ethanol on a fritted-glass filter, air-dried at room temperature overnight, and dried at 100°C. for 8 hours. The dry product was ground in a mortar to a fine powder. For preparation of columns, the CMC was slurried in water, poured into the column, and allowed to settle. The column was given a final rinse with water just before introduction of the sample.

The jacketed chromatographic columns were cooled by tapwater (15° C.). Column flow rates of approximately 1 ml. per 3 minutes were maintained during all operations, except when the sample was introduced. Two-milliliter fractions were collected with an automatic, volume-control collector. The progress of a run was followed by measuring the absorbance of the solutions in the range of 240 to 340 mμ in a 1-cm. cell of a Cary recording spectrophotometer Model 11. Recoveries were based upon the absorbance at 277 mμ, which was the wave length of maximum absorbance for purified samples of the protein. A linear relationship between concentration of absorbing materials and absorbance was assumed.

Experiments and Results

Purification of Extract for Chromatography. A water extract was obtained by stirring 240 g. of meal and 1,200 ml. of water for 1 hour at 1°–2°C. Residual meal was removed by centrifugation. The supernatant solution (about 900 ml.) contained 0.4 mg. of nitrogen and 0.4 mg. of phosphorus per ml. The phosphorus content of the extract was reduced to 10% or less of its original value by two treatments

[3]Mention of trade names or products does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

with 20-g. quantities of moist Dowex-1 (10X) in the acetate form as described earlier (3). Adequate removal of phytate ions was indicated by complete solubility of the protein when the pH of the extract was adjusted to 2.8.

After lyophilization, the resin-treated extract was dissolved in 100 ml. of water, and the pH of the solution was adjusted to 2.6 with 0.5*N* hydrochloric acid. The solution was dialyzed against two 2-liter quantities of 1% acetic acid at 2°C.; the two dialysis periods were held to a minimum by stopping the dialysis when the conductance of the dialysate became constant (e.g., 2.5 and 1.5 hours, respectively). The conductance of the final dialysate was approximately that of 0.0005*M* sodium chloride in 1% acetic acid. After centrifugation to remove slight turbidity, the supernatant solution, containing 1.37 mg. of nitrogen per ml., was frozen and stored at –18°C.

Chromatographic Fractionation. The general procedure for chromatography involved: (a) adsorption of the sample from 1% acetic acid, which provided low ionic strength (about 0.002) combined with low pH (2.8) and high buffering capacity; (b) rinsing the column with 1% acetic acid to remove unadsorbed material; and (c) stepwise elution of adsorbed material with increasing ionic strength at constant pH. Ionic strength was increased by adding sodium chloride to the 1% acetic acid; in all subsequent references to the chromatography the use of 1% acetic acid solutions may be assumed. The column used for the chromatography had an approximate volume of 38 ml. (9 by

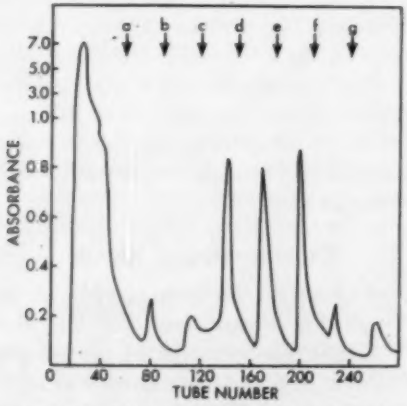

Fig. 1. Fractions obtained by chromatography of purified corn extract applied in 1% acetic acid. After a rinse with 1% acetic acid, elutriants, which were 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, and 0.5*M* sodium chloride in 1% acetic acid, were placed on the column at points a, b, c, d, e, f, and g, respectively, and the absorbance at 277 mμ was measured on the effluent.

610 mm.) and held about 6.5 g. of CMC. Twenty milliliters of the preparation containing 27.4 mg. of nitrogen were placed on the column, which then was rinsed with 60 ml. of 1% acetic acid. Stepwise elutions were made with successive 60-ml. quantities of 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, and 0.5*M* sodium chloride in 1% acetic acid. In Fig. 1 the absorbance at 277 mμ is plotted against the tube number of the fractions. A large portion (58%) of the absorbance came through in the unadsorbed fraction. Although some material was removed with each elutriant, the largest fractions were those removed with elutriants containing 0.05, 0.1, and 0.2*M* sodium chloride. Calculations based on the results in Fig. 1 showed a recovery of approximately 86%. Several replicate chromatographs gave patterns similar to Fig. 1.

Absorption Spectra and Protein Recoveries. In part of these studies the chromatographic separation was modified so that all of the desorbed protein occurred in one fraction. For this purpose the column was eluted with 0.5*M* sodium chloride in 1% acetic acid after removal of the unadsorbed fraction by rinsing with 1% acetic acid. The re-

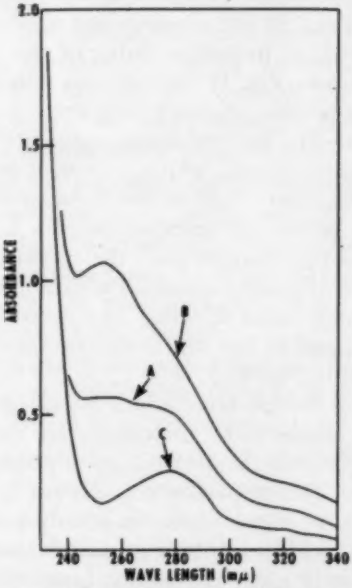

Fig. 2. Absorption spectra: Curve *A* for the purified initial corn extract; curve *B* for the entire unadsorbed fraction from the chromatographic separation; and curve *C* for the adsorbed material eluted in one step by 0.5*M* sodium chloride, corresponding to a combination of the eluted fractions of Fig. 1. All represent the same nitrogen concentration (approximately 0.05 mg. per ml.) — curve *A* as replotted and curves *B* and *C* as observed.

sulting unadsorbed fraction and total desorbed fraction had sufficient concentration of protein to permit determining both nitrogen content and ultraviolet absorption spectrum.

The absorption spectrum curves for the purified initial extract, the unadsorbed fraction, and the total desorbed fraction are shown in Fig. 2. To facilitate comparisons, the absorbance data for the initial extract was replotted so that all the curves in Fig. 2 correspond to the same nitrogen concentration (approximately 0.05 mg. per ml.). Curve *A*, for the preparation of initial extract, has a maximum at 255 mμ, a shoulder at approximately 280 mμ, and appreciable absorption in the range of 300 to 340 mμ. Although there is absorption around 280 mμ as expected of protein solutions, the curve definitely is not typical of purified proteins. The strong absorption in the region of 260 mμ is typical of nucleic acids.

The absorption in the 250–260 mμ range is accentuated in the spectrum of the unadsorbed portion, curve *B*. The maximum is at 252 mμ with only a slight shoulder around 280 mμ. Nitrogen and phosphorus determinations on a comparable unadsorbed fraction before dilution showed 0.58 mg. nitrogen and 0.016 mg. phosphorus per ml. Spectral analysis of individual tubes of the unadsorbed fraction (the initial fraction in Fig. 1) showed some concentrating of nucleic acids in the early portion (tubes 19–33) and of protein in the later portions (tubes 51–61), but no distinct separation. Most of the absorption from 300 to 340 mμ, which is evident in curves *A* and *B* of Fig. 2, occurred in tubes 17–25 of the unadsorbed fraction and corresponded to the yellow pigments of the corn extract.

The total desorbed fraction gave the spectral curve *C* of Fig. 2. It resembles a typical protein spectrum with a minimum at about 250 mμ and a maximum about 277 mμ. The individual tubes and combined fractions eluted in the run shown in Fig. 1 also gave patterns similar to curve *C* in Fig. 2.

The presence of nucleic acids, which have high specific absorbance even at 277 mμ, places some limitations on the use of ultraviolet absorption for measuring the distribution of proteins in the chromatographic fractions. The apparent recovery of protein as measured by absorbance will be higher than the actual recovery when the ratio of nucleic acids to protein is higher in the fraction than in the original mixture, and lower when the ratio is lower. This effect was rather marked, for example, in the gross separation of the corn extract into unadsorbed and total desorbed fractions. On the basis of absorbance at 277 mμ and of nitrogen analysis, respectively, the unadsorbed fraction (higher in nucleic acids) contained 64 and 43% of the initial

material; the total desorbed fraction (lower in nucleic acids) contained 22 and 40%; and the over-all recovery was 86 and 83%.

The relative distribution of protein among individual tubes of the desorbed fractions was indicated with reasonable accuracy by the absorbance at 277 mμ, as demonstrated by a limited number of analyses of column effluents by the colorimetric protein method of Lowry *et al.* (6). In these fractions the interfering effect of nucleic acids is small, since the ratio of nucleic acids to protein is low and probably fairly constant. The absorbance method therefore was adopted for evaluating the chromatographic fractionations. Advantages of the method included retention of the entire sample for further study, and applicability to the small quantities of material in the effluent fractions.

Rechromatography of Fractions. Each of the four major fractions shown in Fig. 1 was rechromatographed. One-half of the unadsorbed fraction was used without further treatment, while each of the three eluted fractions was freed of sodium chloride by a short dialysis against 1% acetic acid, as described for chromatographic fractionation of the extract. The column for rechromatography of the unadsorbed fraction was the same size as for the original fractionation; thus, ample adsorption capacity was ensured. For the three eluted fractions a smaller column was used, having a volume of about 15 ml. (7 by 380 mm.) and holding about 2.5 g. of CMC.

After the fraction was placed on the column, unadsorbed material was displaced by rinsing with 1% acetic acid (50 ml. for the large column and 40 ml. for the small one). For elution, 30 ml. of each elutriant was used. Typical results of rechromatography of the four major fractions (Fig. 1) are given in Fig. 3, where absorbance at 277 mμ is plotted against the tube number.

The originally unadsorbed fraction was not adsorbed on rechromatography (Fig. 3, A). The recovery was approximately 98%, and no peaks were observed in the elution steps.

Rechromatography of the three fractions originally eluted with 0.05*M*, 0.1*M*, and 0.2*M* sodium chloride resulted in Fig. 3, B, C, and D. The main elution peak in each case was obtained at the same sodium chloride concentration as used in eluting the original fraction. Recoveries in the main peak from each of the three fractions were 50, 47, and 35% as measured by absorbance at 277 mμ; in all cases about 17% was eluted in the peak before and about 9% in the peak after the main one. Unadsorbed material was recovered in each of the three rechromatographs (Fig. 3, B, C, and D), accounting for about 20%

of the total original absorbance. Total recoveries from the column were 92, 94, and 83% of the original absorbance.

The unadsorbed fraction had an absorption maximum at about 255 mμ, indicating that the absorbance resulted primarily from nucleic acids. The effectiveness of rechromatography for eliminating nucleic acid contaminants from the main protein components also was demonstrated by calculating the ratio of absorbance at 280 mμ to that at 260 mμ (A280:A260). The fractions before rechromatography had A280:A260 ratios from 1.2 to 1.4. For individual tubes of the main elution peak of the rechromatographs the ratios had in-

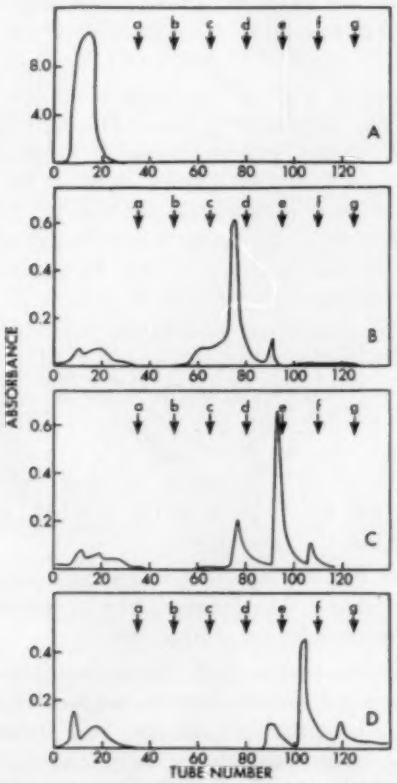

Fig. 3. Rechromatography of fractions obtained by chromatography of purified corn extract applied in 1% acetic acid. After a rinse with 1% acetic acid, elutriants, which were 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, and 0.5*M* sodium chloride in 1% acetic acid, were placed on the column at points a, b, c, d, e, f, and g, respectively, and the absorbance at 277 mμ was measured on the effluent. A, the unadsorbed fraction; B, the fraction eluted with 0.05 *M* sodium chloride; C, the fraction eluted with 0.1*M* sodium chloride; D, the fraction eluted with 0.2*M* sodium chloride.

creased to 1.4 to 1.6. A ratio of 1.75 has been given for highly purified proteins by Taylor (11), whereas for nucleic acids the ratio is about 0.5.

Effect of Prolonged Dialysis on Initial Extract. A lyophilized resin-treated extract prepared as described was dissolved in 50 ml. of 1% acetic acid and dialyzed against several changes of 1% acetic acid at 2°C. for a total of 48 hours instead of the minimum time (about 4 hours). After removal of a slight amount of precipitate by centrifugation, the supernatant solution contained 0.86 mg. of nitrogen per ml. The total nitrogen content of this preparation was only 63% of that obtained with the short dialysis time. The ultraviolet absorption spectrum of the preparation was similar to the protein-type curve, Fig. 2, C, instead of curve *A*.

Discussion

The presence of a proteolytic enzyme in water extracts of corn has been reported previously (3). This protease was found to have a maximum activity at 25°–45°C. at about pH 4.5, with no observed activity at 0°C. at any pH. In the present studies prolonged dialysis (48 hours) of the initial extract resulted in a nitrogen recovery nearly 40% below that obtained on short dialysis (4 hours). This loss could result either from proteolysis or the presence of slowly dialyzing nitrogen compounds. If proteolysis is responsible, the activity of the enzyme at 0°C. might be ascribed to the treatment with the anion-exchange resin, resulting in either an increased solubility of the enzyme or removal of some inhibitors. The results of prolonged dialysis also provided evidence of nuclease activity. The lower absorbance and the change in absorption curve from nucleic acid-type (Fig. 2, A) to nearly protein-type (Fig. 2, C) indicate a high loss of nucleic acids, as a result, presumably, of enzymatic hydrolysis to units small enough to be removed by dialysis. The amount of nucleic acids lost, however, would not account for the decrease in nitrogen recovery on prolonged dialysis. Although a decrease in nucleic acid content of extracts would be advantageous in studies of corn globulins, the accompanying possibility of enzymatic degradation of proteins is objectionable. Since the short dialysis time was effective in preventing extensive nitrogen loss, this procedure was used for preparing samples to determine the success of protein fractionation on CMC.

The two conditions for adsorption of protein on CMC emphasized in this work are low-ionic strength and low pH, attained by using 1% acetic acid as solvent. At low-ionic strength adsorption is enhanced, as previously reported (1,5,9,10) and as confirmed in these studies on corn extracts. This effect presumably results from competition be-

tween protein and inorganic ions for exchange sites; the amount of protein adsorbed increases with decreasing ionic strength. Use of low pH at which the protein exists as a cation further promotes retention of protein on CMC by ion exchange. The extensive elution of protein at high-ionic strength gave a fractionation scheme based upon the variable affinity of the CMC for the different proteins in the mixture. Reproducible chromatographic patterns were obtained by this method. As others have noted (5,9), the affinity between proteins and CMC was low enough to allow recovery of most of the protein. The recovery of material absorbing at 277 $m\mu$ and of nitrogen was in the range of 82–86%.

Evidence that the initial chromatographic fractionation separated protein components or specific protein fractions was provided by rechromatography of the eluted fractions as reported for other naturally occurring mixtures of nitrogenous compounds (7). On rechromatography most of the protein of each fraction appeared at the original elution position of that fraction, with some material in adjacent peaks. In each case the peak preceding the main peak contained a larger portion than the one following it. Tailing of each eluted fraction in the original chromatographic patterns caused portions of the "tail" to occur in the rechromatograph of the succeeding fraction.

Failure of the unadsorbed fraction to be retained on CMC when rechromatographed from 1% acetic acid indicated that it also represented a true fraction and did not result from exceeding the column capacity in the adsorption step. The protein character of the nitrogenous constituents of this fraction was indicated by a high atom ratio of nitrogen to phosphorus (about 80 when compared with 2 to 5 for nucleic acids), although nucleic acids accounted for a large part of the absorbance at 277 $m\mu$ as shown by ultraviolet spectra. Most of the nucleic acids and the yellow pigments in the corn extract remained in the unadsorbed fraction.

The proteins fractionated in this study are only a portion of the globulins of corn. A more complete extraction of the globulins was obtained recently in this laboratory (2) with the use of a carbonate buffer. After methods of completely eliminating possible protease activity have been found, fractionation and quantitative assay of the more complete protein group will be possible with the procedure described here. As the method is simple, gives high recovery, and provides good resolution, its application to other protein mixtures is readily visualized. The ultracentrifuge work of Quensel (8) and Danielson (4) indicates that the same globulins exist in the different

species of the *Gramineae*. This hypothesis could be more adequately established by a study of characteristics other than molecular weight, for example by a combination of chromatography on CMC with electrophoresis.

Acknowledgment

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Literature Cited

1. Boardman, N. K., and Partridge, S. M. Separation of neutral proteins on ion-exchange resins. Biochem. J. **59:** 543–552 (1955).
2. Craine, E. M., and Boundy, Joyce A. The proteins in aqueous salt solution extracts of corn. Abstracts of Papers, Amer. Chem. Soc. **133,** 4A (April 1958).
3. Craine, E. M., and Fahrenholtz, K. E. The proteins in water extracts of corn. Cereal Chem. **35:** 245–259 (1958).
4. Danielson, C. E. Investigations of the seed proteins of the *Gramineae* and *Leguminosae*. Svensk Kem. Tid. **64:** 43–63 (1952).
5. Ellis, S., and Simpson, Miriam E. The chromatography of growth hormone on cellulose derivatives. J. Biol. Chem. **220:** 939–949 (1956).
6. Lowry, O. H., Rosenbrough, Nira J., Farr, A. L., and Randall, Rose J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275 (1951).
7. Moore, S., and Stein, W. H. Column chromatography of peptides and proteins. *In* Advances in protein chemistry, vol. 11, pp. 191–236. Academic Press: New York (1956).
8. Quensel, O. Untersuchungen über die Gerstenglobuline. Dissertation, Uppsala, 1942.
9. Rhodes, M. B., Azari, P. R., and Feeney, R. E. Analysis, fractionation, and purification of egg white proteins with cellulose-cation exchanger. J. Biol. Chem. **230:** 399–408 (1958).
10. Sober, H. A., Gutter, F. J., Wyckoff, Mary M., and Peterson, E. A. Chromatography of proteins. II. Fractionation of serum protein on anion-exchange cellulose. J. Am. Chem. Soc. **78:** 756–763 (1956).
11. Taylor, J. F. The isolation of proteins. The Proteins, Vol. **IA,** pp. 1–85. Academic Press: New York (1953).
12. Truog, E., and Meyer, A. H. Improvements in the Denigès colorimetric method for phosphorus and arsenic. Ind. Eng. Chem., Anal. Ed. **1:** 136–139 (1929).

WET-MILLING HIGH-AMYLOSE CORN CONTAINING 49- AND 57-PERCENT-AMYLOSE STARCH[1]

R. A. ANDERSON, C. VOJNOVICH, and E. L. GRIFFIN, JR.

ABSTRACT

High-amylose corn with starch containing 49 and 57% amylose can be wet-milled, with some modification in processing conditions, to produce a starch of slightly higher protein content, and in somewhat lesser yield, than is usually obtained from ordinary corn. A 64.4% yield of starch containing 0.51% protein was recovered from ordinary corn, as compared with a 43.5% yield of starch containing 0.7% protein from high-amylose corn (57%). An unusually large swelling of the corn kernels was observed during the steeping of high-amylose corn (57%). These kernels exhibited an increase of 128% of their original dry volume, as compared with a 63% increase in volume observed during the steeping of ordinary corn.

Interest in the potential industrial utilization of amylose and high-amylose starches has been increasing over the past few years. Considerable research is being carried out in this field by governmental, educational, and industrial laboratories. Cooperative work of the Northern Utilization Research and Development Division with a commercial corn breeder, and with corn breeders in Production Research, Agricultural Research Service, U. S. Department of Agriculture, stationed at the Agricultural Experiment Station of the University of Missouri, has resulted in the development of corn with starch containing from 50 to 80% amylose, as compared with the 24–27% present in ordinary corn starch (6,11). Generally, the amount of corn available with starch containing above 60% amylose has been limited to small quantities of breeding stock; considerable amounts of field-type corn in the 50–60% range have been grown.

During the wet-milling of a number of small samples of high-amylose corn to recover starch for evaluation studies, processing difficulties were encountered in the separation of starch and gluten. The smaller size and more irregular shape of the high-amylose starch granules made them more difficult to separate from the gluten, resulting in the recovery of starch with a high protein content (2).

This paper discusses the wet-milling characteristics of corn samples with starch containing about 49 and 57% amylose and compares them with those of an ordinary dent corn.

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Materials and Methods

Materials. Samples of high-amylose dent-type corn, from both the 1957 and 1958 crops, were used in these tests. A sample of ordinary dent-type corn grown in 1956 was used for comparison.

Processing Methods. All experiments were first carried out on a laboratory scale and then confirmed in the pilot plant.

In the laboratory experiments, 1,500 g. of corn were steeped in 2,800 ml. of distilled water containing 0.25% sulfur dioxide at 125°F. After 48 hours of steeping, the water was drained, the grain was coarsely ground in a Quaker City[2] drug mill, and the germ was recovered by simple flotation. The fibrous material remaining was finely ground in the drug mill. The resulting slurry was screened first on a 0.039-in. perforated copper sieve to remove coarse fibers and then on a 200-mesh stainless-steel sieve to remove fine fibers. The final slurry, the millstarch, was tabled to separate the starch from the gluten. This procedure has been described by Zipf *et al.* (10).

In the pilot-plant experiments, 2 bu. of grain were steeped in tapwater containing sulfur dioxide. After steeping was completed, the water was drained, and the corn was ground in a Bauer mill equipped with jaw-toothed plates to crack the kernel and free the germ. The germ was recovered by flotation, and the remaining material was ground in a Rietz disintegrator equipped with a 1/16-in. round, perforated screen. Coarse fibers were removed from the slurry by passing it over a Rotex gyratory shaker equipped with a 26-mesh screen; fine fibers were recovered by passing it over the same shaker equipped with a 200-mesh screen. The resulting millstarch was separated into starch and gluten by tabling. The pilot-plant equipment and procedures have been described by Anderson (3).

Analytical Methods. The moisture of the corn and of the various recovered fractions was determined by drying a sample for 4 hours at 110°C., under a vacuum of 28 in. of mercury. Protein ($N \times 6.25$) was determined by an improved Kjeldahl method for nitrate-free samples (1). Starch content was determined polarimetrically by the procedure of Earle and Milner (7). Apparent amylose content of the corn and of the separated starch was determined by the iodine sorption method of Bates, French, and Rundle (5), as modified by Wilson, Schoch, and Hudson (8). The reported amylose figures in the high-amylose corn are generally high, as it has been shown that the amount of material which can be isolated as amylose from this corn is significantly lower (9). Fat analysis was carried out according to the official

[2] Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

method of analysis of the AOAC (4). Sulfur dioxide in the steepwater was determined by titration with iodine solution using starch as the indicator. Solubles and sugar analyses were conducted according to the laboratory methods of AACC (1). All recovery and analytical data presented are on a moisture-free basis (M.F.B.).

Results and Discussion

Establishment of Optimum Conditions and Procedure. The described general processing procedure for wet-milling cereal grains has been established over the years by the performance of many experiments on corn, wheat, and grain sorghum. After several samples of high-amylose corn had been milled it was found that some modification had to be made. In particular, a change in the starch-gluten separation was needed, since a good recovery of high-purity starch could not be obtained.

In the investigation of the starch-gluten separation, variables studied were the pitch of the starch table, specific gravity of the millstarch, and the effect of double tabling (Table I). The effect of increasing the table pitch, while the other variables remained constant, was to improve the purity of the starch. When the millstarch was tabled at a pitch of ½ in. per 20 ft., the protein content of the resulting starch was 4.3%. There was substantial improvement in the purity of the starch, with little sacrifice in recovery, when the millstarch was tabled at a table pitch of 1 in. per 20 ft. Raising the table to 2 in. per

TABLE I

EFFECT OF CERTAIN VARIABLES ON THE STARCH-GLUTEN SEPARATION OF HIGH-AMYLOSE CORN (49%)

VARIABLE	PITCH OF TABLE			SPECIFIC GRAVITY OF MILLSTARCH		DOUBLE TABLING OF STARCH	
Specific gravity of millstarch, ° Baumé at 60°F.	3.5	3.5	3.5	3.5	6.0	3.5	3.5
Pitch of table, in./20 ft.	½	1	2	2	2	1	2
Recovery of starch,[a] % M.F.B.[b]	88.2	85.1	83.6	83.6	83.2	...	80.0
Purity of recovered starch, % protein, M.F.B.	4.3	1.33	1.05	1.05	1.12	...	0.53
Purity of recovered gluten, % protein, M.F.B.	33.4	37.5	33.1	33.1	33.5	32.2	...

[a] Recovery of starch based on amount of starch present in corn.
[b] Moisture-free basis.

20 ft. resulted in still more improvement in the starch purity, while maintaining a good starch recovery. As the pitch of the table is increased, the smaller starch granules go off the table with the gluten and the protein content of the gluten remains about the same.

Variation of the specific gravity of the millstarch between 3.5° and 6.0° Baumé at 60°F. caused only minor differences in the starch purity and recovery. However, at 3.5° Baumé a slightly better starch was recovered, and this specific gravity was selected as optimum for the remaining work.

Starch of good purity was recovered by tabling the millstarch twice. The first tabling was carried out at a table pitch of 1 in. per 20 ft. Gluten containing 32.2% protein was recovered from this table. The starch was then reslurried and retabled at a pitch of 2 in. per 20 ft., yielding starch with a protein content of 0.53%. Starch recovery by double tabling was about 5% less than obtained by single tabling. The overflow from the second tabling, the squeegee starch, had a protein content of about 17%. Double tabling results in obtaining a good grade of starch with fair recoveries from high-amylose corn containing starch having 49% amylose.

Comparison of Processing Characteristics of High-Amylose and Ordinary Dent-Type Corn. Processing characteristics of two samples

TABLE II

COMPOSITION OF CORN USED IN WET-MILLING EXPERIMENTS
(Moisture-free basis)

COMPOSITION	ORDINARY CORN	HIGH-AMYLOSE CORN (49%)	HIGH-AMYLOSE CORN (57%)
Crop year	1956	1957	1958
Apparent amylose content of starch, %	24	49	57
Protein, %	10.1	12.6	13.9
Fat, %	4.5	6.7	7.1
Starch, %	73.8	62.5	60.9
Solubles, %	5.6	7.3	8.6
Total sugars, %	2.3	3.2	2.6

of high-amylose corn and a sample of ordinary corn were compared on a laboratory scale. The composition of the different corn samples is given in Table II. The protein, oil, and solubles content of the corn increased and the starch content decreased as the amount of amylose present in the starch became greater.

Steeping characteristics of the samples showed some differences when the steeping of the corn was followed for the complete steeping time. This was accomplished by analyzing, at intervals, the steeped

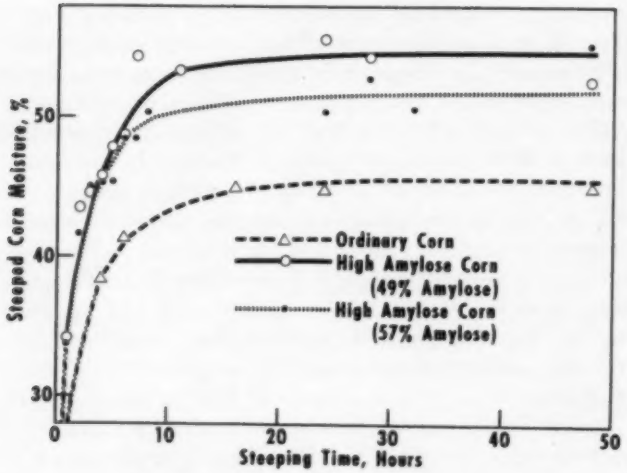

Fig. 1. Effect of steeping time on the steeped corn moisture.

corn for moisture and also the steepwater for pH and sulfur dioxide content. The steeped corn moisture curve from high-amylose corn followed the characteristic water absorption curves obtained from the steeping of ordinary corn, as shown in Fig. 1. However, the high-amylose corn absorbed from 6 to 8% more water in about the same amount of time as did the ordinary corn. As illustrated in Fig. 2, the pH of the steepwater obtained from the three corn samples followed similar curves, except that the pH of the steepwater from ordinary

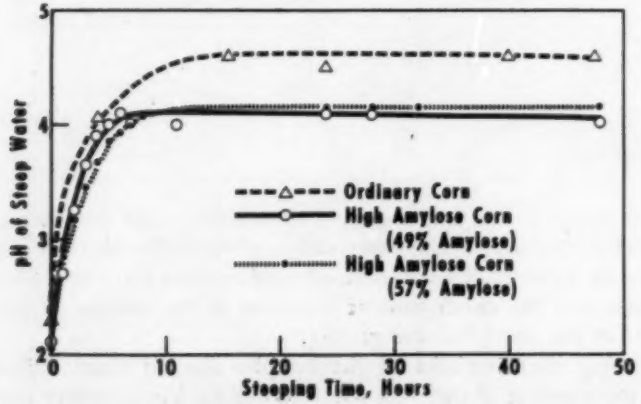

Fig. 2. Effect of steeping time on the pH of the steepwater.

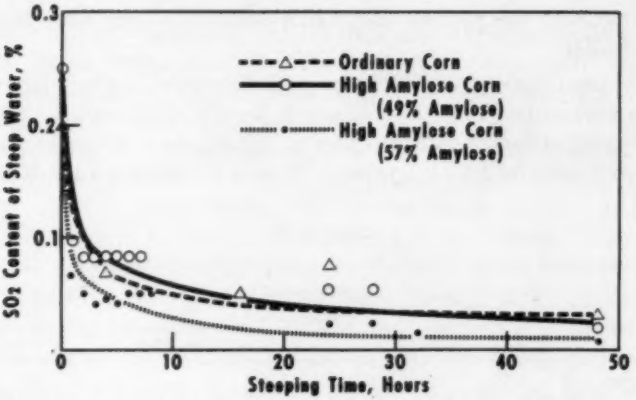

Fig. 3. Effect of steeping time on the sulfur dioxide content of the steepwater.

corn was somewhat higher than from high-amylose corn. The disappearance of sulfur dioxide from the steepwater was about the same for the three samples (Fig. 3). The yield of solids and their protein content in the steepwater were about the same for all three samples, averaging 4.3 and 30.8%, respectively. This comparison and the comparison of other processing characteristics of the three different corn samples are given in Table III. The amount of reducing sugars in the steepwater varied from 0.76% from ordinary corn steepwater to about 2% in the steepwater from high-amylose corn.

One significant difference was the amount of swelling of the high-amylose corn kernel as compared with ordinary corn. During steeping, high-amylose corn (57%) exhibited an increase of 128% of its original dry volume, whereas high-amylose corn (49%) had an increase of 100%, and ordinary corn only 63%. This degree of swelling would decrease the amount of high-amylose corn that could be steeped in tanks of a given size, which might force a reduction in the daily grind in a commercial plant.

Germ yield from the processing of both samples of high-amylose corn amounted to 6.5%, whereas germ yield from ordinary corn was 4.7%. This increase is undoubtedly due to the larger germ present in high-amylose corn. Oil content of the germ was about the same for the three samples; however, recovery of total oil from the high-amylose corn germ was slightly less than from ordinary corn – 51% as compared with 55.5%.

There were no significant differences in the manner in which the coarse or fine fibers from either high-amylose or ordinary corn responded during processing. The amount of fibers recovered was about

the same, and the protein and starch contents of the fiber fractions were similar.

The most significant differences were observed during the starch-gluten separation. The yield of starch from a single tabling of mill-starch from ordinary corn was 64.4% (87.3% on a starch basis), and the starch contained 0.51% protein. This is an optimum result for the

TABLE III

COMPARISON OF THE PROCESSING OF THREE DIFFERENT SAMPLES OF CORN

	ORDINARY CORN	HIGH-AMYLOSE CORN (49%)	HIGH-AMYLOSE CORN (57%)
Steepwater			
Yield[a] of solids, % M.F.B.[b]	4.2	5.1	3.6
Protein content, % M.F.B.	31.2	30.4	31.0
Reducing sugars, % M.F.B.	0.76	1.9	2.0
Steeped corn			
Average final moisture, %	46	54	52
Volume increase, %	63	100	128
Germ			
Yield, % M.F.B.	4.7	6.5	6.5
Crude oil content, % M.F.B.	53.0	52.9	56.0
Recovery of total oil, %	55.5	51.4	51.0
Coarse fiber			
Yield, % M.F.B.	12.8	11.1	12.6
Protein content, % M.F.B.	14.9	12.7	10.4
Starch content, % M.F.B.	19.8	12.4	12.4
Fine fiber			
Yield, % M.F.B.	6.2	6.4	4.9
Protein content, % M.F.B.	23.8	22.2	21.1
Starch content, % M.F.B.	30.6	31.8	30.1
Starch			
Yield, % M.F.B.	64.4	50.0	43.5
Recovery,[c] %	87.3	80.0	71.4
Protein content, % M.F.B.	0.51	0.53	0.7
Gluten			
Yield, % M.F.B.	7.8	5.3	15.5
Protein content, % M.F.B.	44.2	30.0	33.1
Starch content, % M.F.B.	....	52.7	52.3
Squeegee starch[d]			
Yield, % M.F.B.	....	3.5	4.9
Protein content, % M.F.B.	....	18.9	5.0
Starch content, % M.F.B.	....	71.6	94.4
Process waters			
Yield of solids, % M.F.B.	2.5	6.1	6.9
Protein content, % M.F.B.	40.0	37.0	44.5

[a] Yield based on original weight of dry corn.
[b] Moisture-free basis.
[c] Recovery of starch based on starch present in corn.
[d] Squeegee starch is result of second tabling.

general batch procedure followed in these studies. It would be expected that better results would be obtained in a commercial operation using countercurrent steeping and centrifuges or hydroclones for the starch-gluten separation.

In contrast, double tabling of millstarch from high-amylose corn, both the 49 and 57% variety, resulted in lower yields, 50 and 43.5%, respectively. This loss is partially due to the smaller amount of starch originally present in high-amylose corn, but is probably caused primarily by the presence of small and irregularly shaped starch granules. On the basis of experience with ordinary corn, it seems improbable that the higher protein content of high-amylose corn is an appreciable factor affecting the starch-gluten separation. The loss is further emphasized by the amount of high-amylose starch recovered, based on the starch in the corn. The starch recovery from 49%-amylose corn was 80.0%, and from the 57% material only 71.4% was recovered. The 0.53% protein content of the starch recovered from the 49%-amylose corn compares favorably with starch from ordinary corn. However, the starch obtained from 57%-amylose corn contained slightly more protein (0.7%); this, coupled with its lower yield, illustrates the difficulty involved in separating starch and gluten from corn with an increased amylose content.

The yield and protein content of the gluten recovered from the ordinary corn were 7.8 and 44.2%, respectively. This is of the magnitude usually obtained for a commercial tabling operation. The yield of glutens from high-amylose corn varied from 5.3 to 15.5%. They contained only 30 to 35% protein and over 50% starch. The "squeegee" starch, which was overflow or "gluten" from the second tabling of high-amylose starch, had very high starch contents, as expected.

The solids present in the process waters from wet-milling ordinary corn amounted to 2.5% of the corn processed and contained 40% protein. The process waters from high-amylose corn, 49 and 57%, contained 6.1 and 6.9% solids, respectively, and their protein contents also averaged about 40%. The recovery of a greater quantity of solids in process waters from the two high-amylose corns can be attributed to their higher soluble content and also to the presence of very small starch granules which did not settle out in the various settling operations.

Two-bushel lots of each of the three corn samples were processed in the pilot plant to confirm the data and results obtained in the laboratory studies. The observations made during the larger-scale runs were quite similar to those made in the laboratory. While starch recovered from ordinary corn had a protein content of 0.47%, starch

from both the 49- and 57%-amylose corn contained about 0.6% protein.

Similar comparative investigations will be made as plant breeders provide samples of corn that contains starch with even higher percentages of amylose than corn used in this study.

Acknowledgment

The high-amylose corn for these studies was made available through the courtesy of the American Maize-Products Co., Roby, Indiana, and National Starch Products, Inc., Plainfield, N. J. The two samples are from strains developed by the Bear Hybrid Corn Company, Decatur, Illinois. The authors are grateful to J. G. Pemberton and L. H. Burbridge for their assistance in carrying out the experimental work, to D. H. Johnson for analyzing the many samples, and to W. L. Deatherage for the amylose analysis of the corn.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. Anderson, R. A., and Pfeifer, V. F. A note on experimental wet-milling of high-amylose corn. Cereal Chem. **36:** 98–101 (1959).
3. Anderson, R. A. A pilot plant for wet-milling. Cereal Sci. Today **2:** 78–80 (1957).
4. Association of Official Agricultural Chemists. Official methods of analysis (8th ed.). The Association: Washington, D.C. (1955).
5. Bates, F. L., French, D., and Rundle, R. E. Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. **65:** 142–148 (1943).
6. Deatherage, W. L., MacMasters, Majel M., Vineyard, M. L., and Bear, R. P. A note on starch of high-amylose content from corn with high starch content. Cereal Chem. **31:** 50–52 (1954).
7. Earle, F. R., and Milner, R. T. Improvements in the determination of starch in corn and wheat. Cereal Chem. **21:** 567–575 (1944).
8. Wilson, E. J., Jr., Schoch, T. J., and Hudson, C. S. The action of *macerans* amylase on the fractions from starch. J. Am. Chem. Soc. **65:** 1380–1383 (1943).
9. Wolff, I. A., Hofreiter, B. T., Watson, P.R., Deatherage, W. L., and MacMasters, Majel M. The structure of a new starch of high amylose content. J. Am. Chem. Soc. **77:** 1654–1659 (1955).
10. Zipf, R. L., Anderson, R. A., and Slotter, R. L. Wet-milling of grain sorghum. Cereal Chem. **27:** 463–476 (1950).
11. Zuber, M. S., Grogan, C. O., Deatherage, W. L., Hubbard, J. E., Schulze, W. E., MacMasters, Majel M. Breeding high-amylose corn. Agronomy J. **50:** 9–12 (1958).

THE BROMATE REACTION IN DOUGH
II. Inhibition and Activation Sudies[1]

W. Bushuk and I. Hlynka

ABSTRACT

The amperometric titration method was used to study effects of seventeen different chemicals on the bromate reaction in dough. Results indicate that: 1) Iodate, chlorine dioxide, and oxygen react with the same reactive components as bromate but at much greater rates and, accordingly, inhibit both phases of the bromate reaction; 2) N-ethylmaleimide, and copper(II), mercury(II), silver and selenite ions inhibit the bromate reaction probably because of their ability to react with sulfhydryl groups; 3) vanadate and versene activate the bromate reaction; the former probably acts as a normal catalyst whereas the latter probably removes inhibiting ions; 4) urea and guanidine hydrochloride have no effect on the bromate reaction, indicating that the rate of the linear reaction is probably not diffusion-controlled.

This paper, the second of the present series (1) on the bromate reaction in dough, continues the kinetic studies by examining the effects of inhibiting and activating reagents. The reagents were selected because they either affect the physical properties of dough or are known to interact with protein sulfhydryl groups which are believed to be involved in the bromate reaction.

Materials and Methods

The flour used in this study was described previously (1). The chemicals used as inhibitors or activators and the grade or origin are listed in Table I.

The method for following the disappearance of the bromate ion in dough was described in the first paper (1). All the experiments reported in this paper were made with initial bromate concentration of 18.75 mg. per kg. of dough (30 p.p.m. of flour) mixed to 60% absorption. The doughs were mixed in nitrogen with the exception of the experiments on the effect of oxygen. To study the effect of oxygen, the flours were first stored overnight under air (20% oxygen) or under pure oxygen, and the doughs were mixed under corresponding atmospheres. Treatment of flour with chlorine dioxide was made by the acetic anhydride method of Parker and Fortmann (8).

In experiments with solid reagents, the desired amount of the reagent, in aqueous solution, was added to the liquid used to mix the dough. The bromate solution was added to the same liquid.

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TABLE I

LIST OF CHEMICALS USED

CHEMICAL	GRADE OR ORIGIN
Oxidizing agents	
Potassium iodate	Reagent
Chlorine dioxide	Prepared in laboratory (8)
Oxygen	Canadian Liquid Air
Sulfhydryl-blocking agents	
N-ethylmaleimide	Nutritional Biochemicals, Inc.
Iodoacetic acid	Eastman Organic Chemicals
Sodium iodoacetate	Eastman Organic Chemicals
Inorganic ions	
Copper(II) chloride	Reagent
Silver nitrate	Reagent
Mercury(II) chloride	Analytical
Calcium chloride	Reagent
Lead nitrate	Reagent
Selenious acid	Reagent
Sodium vanadate (meta)	C.P.
Ammonium molybdate	Analytical
Complexing agent	
Versene (disodium salt of ethylene diamine tetraacetic acid)	Analytical
H-bond-breaking agents	
Urea	Reagent
Guanidine hydrochloride	Reagent

Blank titrations of extracts from doughs, which contained the inhibitor but no bromate, served as controls. The possibility of direct reaction between the added reagent and the bromate ion was checked for reagents which were suspected of such a reaction.

Results and Discussion

The effects of some seventeen different chemicals on the bromate reaction in dough were studied. To simplify the presentation of results, it is convenient to discuss the various reagents in groups according to their type or function as indicated in Table I. Results for each group will be discussed separately.

As in the earlier paper (1), the bromate reaction in dough will be characterized by the amount of initial reaction and the rate of the secondary or linear reaction. Effects on both reactions will be considered.

Oxidizing Agents. The effects on bromate reaction of three oxidizing agents commonly used for improving the baking quality of flour, namely, potassium iodate, chlorine dioxide, and oxygen, were studied. Figure 1 compares the results that were obtained. The upper curve

for each reagent shows the effect on the initial reaction; the bottom curve gives the corresponding effect on the secondary or linear reaction. It is apparent that the oxidizing agents used are strong inhibitors of both phases of the bromate reaction in dough.

Control experiments with potassium iodate showed that, up to about 18.75 mg. per kg., this improver reacts completely in the normal dough during the mixing stage. At higher concentrations small amounts of iodate were recovered; the bromate recoveries for the high iodate concentrations were accordingly corrected for residual iodate which titrates as bromate.

It was not possible to produce 100% inhibition by iodate, even with relatively high concentrations of the inhibitor. This may result from a competitive mechanism in which an equilibrium is established between the reactants and the inhibitor, or it may be due to magnification of experimental errors when bromate recoveries are corrected for large amounts of residual iodate.

In the experiments with chlorine dioxide-treated flour it was observed that if the flour was used immediately after treatment a small amount of reactant, presumably unreacted chlorine dioxide which titrates as bromate, can be detected in control doughs. This residual chlorine dioxide seems to react rapidly after mixing, since no chlorine dioxide could be extracted from the dough after a reaction time of 1 hour. No chlorine dioxide could be recovered immediately after mixing if the flour was stored for several days after treatment.

A comparison of the efficiency of iodate and chlorine dioxide as inhibitors of the bromate reaction on an equivalent basis (assuming that iodate and chlorine dioxide are reduced to the corresponding halide ion) shows that iodate is apparently more efficient. However, this may be because part of the chlorine dioxide reacts in the bleaching reaction. The treated flour was considerably whiter than the original flour.

Figure 1 shows also that oxygen is a highly efficient inhibitor of both phases of the bromate reaction. These results are apparently different from those of Cunnigham and Hlynka (2), who showed that oxygen seems to increase the amount of initial reaction and inhibits only the linear reaction. The reason for this discrepancy is not known.

Sulfhydryl-Blocking Agents. Of the various sulfhydryl-blocking agents that are available, N-ethylmaleimide (NEMI) was selected because it seems to react specifically with sulfhydryl groups (3,9); it affects dough in a way similar to that of iodate; and it is conveniently soluble and adequately stable at the normal pH of dough. Figure 2 compares the inhibition of the bromate reaction by NEMI and iodate.

It is apparent that the two agents are quite similar in their effects on the bromate reaction, although they are quite different chemically. With NEMI, as with iodate, it was impossible to produce 100% inhibition.

The effect of iodoacetic acid or its sodium salt, which may also be classified as a sulfhydryl-blocking agent, was not as clear-cut as that of

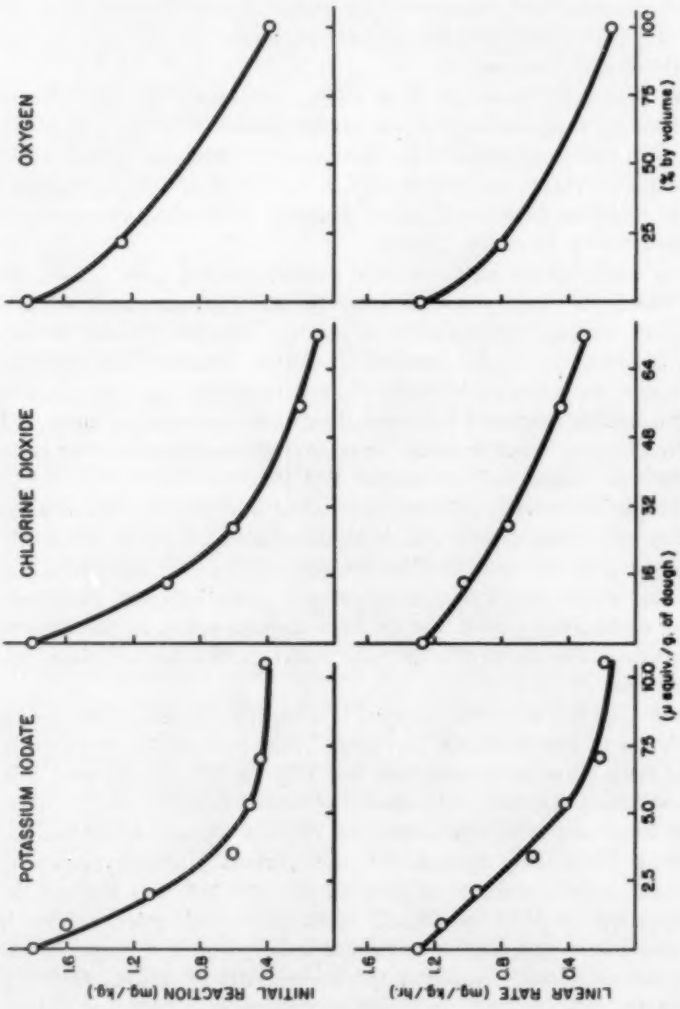

Fig. 1. Inhibition of the bromate reaction in dough by iodate, chlorine dioxide, and oxygen. Upper curves, initial reaction; lower curves, linear reaction.

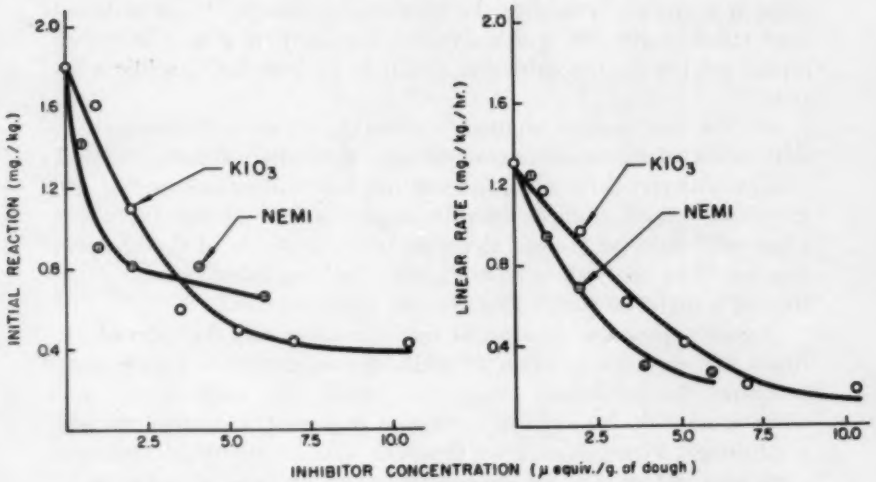

Fig. 2. Comparison of the inhibition of the bromate reaction in dough by N-ethylmaleimide with the inhibition by iodate. Upper curves, initial reaction; lower curves, linear reaction.

any of the inhibitors discussed so far. The maximum inhibition that could be produced with these inhibitors was only about 20%; this maximum occurred at an inhibitor concentration of 150 μmoles per kg. of dough. Increasing the inhibitor concentration further did not increase the amount of inhibition. In the experiments with sodium iodoacetate, an apparent reversal in inhibition was actually obtained. It seems that the results with iodoacetic acid and its sodium salt are complicated by some additional reaction. For example, if hydrogen iodide is produced, it may subsequently react as a reducing agent and consequently counteract the effect of the initial inhibition.

Inorganic Ions. Some inorganic cations show very marked effects on the physical properties of dough. Among these, copper(II), silver, and mercury(II) ions are probably the most active. The mechanisms of these effects are still not known, although it is suspected that the sulfhydryl group may be involved, either through oxidation or mercaptide formation. It was therefore of fundamental interest to study the effects of these ions on the bromate reaction. The effects of two less active cations, calcium and lead, were studied also.

Experiments were also made with three anions. Selenite ion was used because it seems to be a strong catalyst for the oxidation of the sulfhydryl group in glutathione, but is only a weak inhibitor of some sulfhydryl-containing enzymes (10,11); vanadate was used be-

cause it is known to catalyze the bromate reaction in dough as measured rheologically (6); and molybdate was used because it is an accepted catalyst for the oxidation of iodide by bromate in acidic solution.

Of the five cations studied, copper(II), silver, and mercury(II) ions inhibited the bromate reaction very markedly; calcium and lead ions showed very little effect and will not be discussed any further. Of the three anions studied, selenite showed a very strong inhibitive effect; vanadate ion seemed to accelerate both phases of the bromate reaction; and molybdate had no effect on the initial reaction but showed a slight inhibitive effect on the linear reaction.

Figure 3 gives the amounts of initial reaction and the rates of the linear reaction as a function of inhibitor concentration. Figure 3, A, compares the inhibition curves for copper (II), mercury (II), and silver ions, with the ion concentrations expressed in equivalents per g. of dough. Figure 3, B, gives the same data for the silver and mercuric ions, except that the ion concentrations are now in moles per g. This comparison assumes that the reacting ion for mercury (II) chloride is $HgCl^+$ and not Hg^{++} as is implied in Fig. 3, A. It is not known definitely which ion is the active one in dough. Experiments in which sodium chloride was added in conjunction with mercury(II) chloride showed that the additional chloride ion had very little effect on the amount of inhibition. Accordingly, it seems that $HgCl^+$ might be the reacting ion. Figure 3, C, gives the inhibition curves for the selenite ion.

The results (Fig. 3) show that the inhibition mechanism is essentially the same for the four ions. Again the mechanism appears to be competitive. The differences in the degree of inhibition shown in Fig. 3, A and B, are not considered as highly significant, except for the inhibition of the initial reaction by copper(II), in which case 100% inhibition was obtained with fairly low concentrations of the ion. Selenite ion seems to be the strongest inhibitor of the bromate reaction of all the inhibitors studied so far. Of the two ions, silver and mercury(II), silver seems to be a stronger inhibitor on the equivalent basis. On a molar basis, $HgCl^+$ appears to show a slightly higher inhibitive effect on the initial reaction.

It may be speculated as to why only copper(II) can inhibit the initial reaction 100%. One possible explanation that can be advanced is the ability of this ion to catalyze the oxidation of sulfhydryl groups in addition to its ability to form mercaptides or fairly stable complexes. In view of the fact that it is somewhat difficult to recover all the bromate from dough, either as bromide or bromate (5),

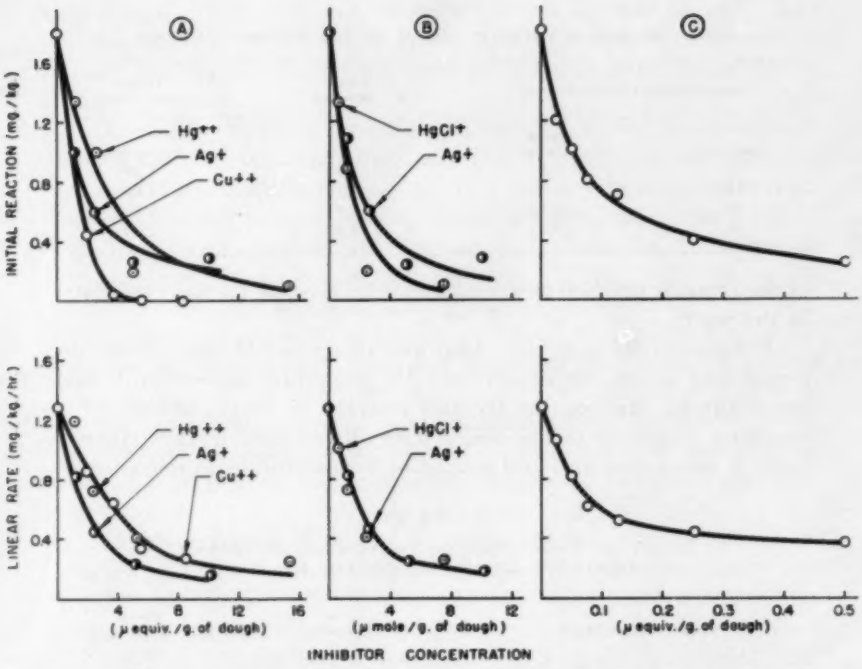

Fig. 3. Inhibition of the bromate reaction in dough by various inorganic ions. A, copper(II), mercury(II), and silver, with ion concentrations in μequiv. per g. of dough; B, mercury(II) chloride and silver nitrate with concentrations in μmoles per g. of dough; C, selenite ion. Upper curves, initial reaction; lower curves, linear reaction.

it is impossible to attach too much significance to the fact that copper-(II) ion can cause 100% inhibition of the initial reaction, whereas most of the other inhibitors can only produce 80 to 90% inhibition.

Table II gives the amounts of initial reaction and the rates of the linear reaction for the disappearance of the bromate ion in four doughs containing 0, 6.25, 12.50, and 18.75 mg. per kg. of sodium meta vanadate. The positive catalytic effect is quite apparent. These findings confirm the conclusions on the effect of vanadate on the bromate reaction based on studies of physical dough properties (6).

Complexing Agents. Table III gives the amounts of initial reaction and the rates of the linear reaction for doughs containing 0, 25, and 50 mg. per kg. of versene. These data show that versene has an apparent positive catalytic effect. This effect is probably due to the ability of versene to complex certain ions, some of which may be inhibitors

TABLE II

EFFECT OF SODIUM VANADATE (META) ON THE BROMATE REACTION

Sodium Vanadate Concentration		Initial Reaction	Linear Reaction
ppm flour	*mg/kg of dough*	*mg/kg*	*mg/kg/hour*
0	0.0	1.80	1.28
10	6.25	3.26	1.65
20	12.50	3.95	1.85
30	18.75	4.80	2.00

of the bromate reaction as was suggested by Hlynka (4) and confirmed in this study.

H-Bond-Breaking Agents. Additions of up to 6*M* urea (based on liquid used to mix the dough) and 4*M* guanidine hydrochloride had essentially no effect on the bromate reaction in dough, although the handling properties of the dough were altered considerably. Accordingly, it seems that urea and guanidine hydrochloride do not uncover

TABLE III

EFFECT OF VERSENE (DISODIUM SALT OF ETHYLENE DIAMINE TETRAACETIC ACID) ON THE BROMATE REACTION

Versene Concentration		Initial Reaction	Linear Reaction
ppm flour	*mg/kg of dough*	*mg/kg*	*mg/kg/hour*
0	0	1.80	1.28
40	25	2.36	1.57
80	50	3.18	1.84

any additional reactive groups and that the rate of the linear reaction, despite the magnitude of the activation energy (1), is not a diffusion-controlled process.

General Discussion

Although the iodate and the bromate ions have essentially the same chemical properties, the rates at which they affect physical dough properties differ widely. Extensigraph studies show that iodate reacts almost entirely during mixing, so that its effect on the physical properties is perceptible immediately, whereas bromate requires a definite reaction time to show its effect. Results reported in this paper suggest that iodate and bromate react with the same group(s) in flour, except that iodate reacts much faster. A difference in the reaction rates would be sufficient to account for the inhibitive effect of iodate on the bromate reaction. It is actually possible to slow down the iodate reaction

in dough considerably by lowering the temperature to 10°C. and raising the pH of the dough to 8.7. The mechanism of chlorine dioxide and oxygen inhibition is apparently the same as that of the iodate inhibition.

The ability of the other chemicals, namely, NEMI, iodoacetic acid and its sodium salt, copper(II), silver, mercury(II) and selenite ions, to inhibit the bromate reaction seems to parallel their ability to react with sulfhydryl groups. Indeed, NEMI is considered to be a highly specific reagent for sulfhydryl groups (3,9). Accordingly, it appears that at least 80 to 90% of the bromate reaction (both phases) in dough occurs with free sulfhydryl groups. The remaining 10 to 20% of the bromate disappearance may represent experimental error involving a physical loss inherent in the reaction system and the method of extraction of the unreacted bromate ion from dough. It should be mentioned in this connection that Matsumoto and Hlynka (7) found that NEMI decreased the sulfhydryl content of water-soluble components of flour by only 62% and of the acid-soluble components by only 40%.

Results reported in this paper add to the weight of evidence, obtained in this and other laboratories, that sulfhydryl is the main group involved in the bromate reaction in dough. Nevertheless, clear-cut results relating sulfhydryl content and disappearance, with disappearance of bromate have not yet been obtained, and the stoichiometry of the over-all reaction remains elusive.

Literature Cited

1. Bushuk, W., and Hlynka, I. The bromate reaction in dough. I. Kinetic studies. Cereal Chem. **37:** 141–150 (1960).
2. Cunningham, D. K., and Hlynka, I. Flour lipids and the bromate reaction. Cereal Chem. **35:** 401–410 (1958).
3. Gregory, J. D. The stability of N-ethylmaleimide and its reaction with sulfhydryl groups. J. Am. Chem. Soc. **77:** 3922–3923 (1955).
4. Hlynka, I. The effect of complexing agents on the bromate reaction in dough. Cereal Chem. **34:** 1–15 (1957).
5. Lee, C. C., Tkachuk, R., and Finlayson, A. J. The conversion of Br^{82}-labeled bromate to bromide in nonfermenting and fermenting doughs and in baked bread. Cereal Chem. **35:** 337–349 (1958).
6. MacIntosh, C. H. Dough improver for leavened bakery products. U.S. Patent 2,095,992 (1937); Chem. Abstr. **32:** 261 (1938).
7. Matsumoto, H., and Hlynka, I. Some aspects of the sulfhydryl-disulfide system in flour and dough. Cereal Chem. **36:** 513–521 (1959).
8. Parker, H. K., and Fortmann, K. L. Methods for the laboratory-scale production of chlorine dioxide and the treatment of flour. Cereal Chem. **26:** 479–490 (1949).
9. Roberts, E., and Rouser, G. Spectrophotometric assay for reaction of N-ethylmaleimide with sulfhydryl groups. Anal. Chem. **30:** 1291–1292 (1958).
10. Tsen, C. C., and Collier, H. B. Selenite as a relatively weak inhibitor of some sulfhydryl enzymes. Nature **183:** 1327–1328 (1959).
11. Tsen, C. C., and Tappel, A. L. Catalytic oxidation of glutathione and other sulfhydryl compounds by selenite. J. Biol. Chem. **233:** 1230–1232 (1958).

THE EFFECT OF REMIXING ON THE STRUCTURAL RELAXATION OF UNLEAVENED DOUGH[1]

A. H. Bloksma[2] and I. Hlynka

ABSTRACT

If unleavened doughs are tested in the Brabender extensigraph according to the structural relaxation procedure, they yield hyperbolic structural relaxation curves irrespective of whether they are or are not remixed before shaping. However, remixing causes a decrease in the structural relaxation constant and in the asymptotic load. The effect increases with increasing duration of remixing. The effect on the structural relaxation decreases when the time interval between remixing and shaping increases from 3 to 20 minutes; this is called recovery. Recovery may or may not be complete in 20 minutes, but under the conditions of the present experiments it does not proceed beyond that time interval.

Doughs to which 35 p.p.m. N-ethylmaleimide are added are least sensitive to remixing, closely followed by doughs with 12.5 p.p.m. iodate. Doughs with 20 p.p.m. bromate and doughs without additions but mixed in an atmosphere of oxygen show a large effect of remixing. With bromated doughs the effect of remixing increases considerably with reaction time. After recovery the rank order of doughs with different additions has remained essentially the same. Recovery of doughs with N-ethylmaleimide is even complete for reaction times shorter than 60 minutes. Doughs mixed in an oxygen atmosphere and remixed in nitrogen hardly show any recovery; if the remixing is done in an oxygen atmosphere, however, recovery is considerable.

Results obtained with the Brabender and Halton extensigraphs show that at least part of the effect of remixing is due to interference with subsequent structural activation during shaping of the test piece. Possibly differences between flour improvers that change rheological dough properties rapidly and slowly are more important than differences between oxidizing and thiol-blocking reagents.

Mechanical work on dough such as mixing, punching, dividing, or molding usually results in a change in its mechanical or rheological properties. Irrespective of the direction in which these properties have changed, the original properties are restored or recover gradually. The time intervals that are necessary for recovery have implications for the minimum time intervals between successive operations in practical breadmaking. These phenomena of change and recovery are illustrated at a more basic level by structural activation and relaxation (10). A dough can also stand a longer mixing time in the farinograph if periods of mixing alternate with periods of rest than if mixing is done continuously; the breakdown during mixing is reversed or

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compensated for during rest. The molecular processes that underlie these phenomena are unknown.

In the present study the change in rheological properties of unleavened doughs caused by remixing was investigated, with particular attention to the restoration of original properties, or recovery, after remixing. Remixing of yeasted doughs at the end of the fermentation period eliminates the adverse effect of excess bromate on loaf properties (12). In the present work other oxidizing flour improvers such as iodate and oxygen were also included, as well as the thiol-blocking reagent N-ethylmaleimide that does not possess oxidizing properties.

The structural relaxation method using the Brabender extensigraph has been useful in studies of flour improvement (5,7–10). In this method a series of test pieces with identical history is shaped and clamped in extensigraph sample holders, and stretched after various rest periods. A curve of the load at an arbitrary but constant extension vs. rest period is called structural relaxation curve; structural relaxation follows structural activation brought about by rounding and shaping the test piece. The effect of the history of the series on the structural relaxation curve provides information on processes that occur before shaping. This method proved to be equally useful for a study of remixing.

Changes in dough properties with increasing *duration of remixing* are reflected in a decrease in the parameters that describe the structural relaxation curves, the relaxation constant, and the asymptotic load. Using a constant duration of remixing, the influence of the *time interval between remixing and shaping* was studied. The decrease in structural relaxation constant as a consequence of remixing is largest for short time intervals between remixing and shaping; it becomes smaller with increasing time intervals up to about 20 minutes, when recovery appears to attain its maximum value. This observation made it possible to compare easily the maximum effect of remixing and the maximum recovery for doughs with different *flour improvers* and after different *reaction times,* by choosing the time intervals between remixing and shaping as short as possible and 20 minutes, respectively. In order to learn more about the interaction between remixing and structural activation, some experiments were done with a load-extension meter in which structural activation during shaping of the test piece could be minimized; for this purpose the Halton extensigraph was chosen.

Materials and Methods

A commercially milled, straight-grade, untreated flour from Cana-

dian hard spring wheat was used. It contained 13.2% protein (N × 5.7) and 0.46% ash on a 14% moisture basis. The farinograph water absorption was 64.5%.

Doughs were prepared from 200 g. flour (14% moisture basis), 2 g. sodium chloride, 114.2 g. (57.1%) water, with or without additions of potassium bromate, potassium iodate, or N-ethylmaleimide (NEMI). Flour was stored overnight in an atmosphere of nitrogen before use. Salts were reagent grade, and N-ethylmaleimide was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

The procedure used is the structural relaxation method for the Brabender extensigraph, as described earlier (9). The essential modification is that, except in control experiments, part of the mixing was done after a portion of the reaction time had elapsed. This second part of mixing, or remixing, divided the reaction time into two intervals: the first interval from the end of initial mixing to the end of remixing, and the *second interval* from the end of remixing until the end of shaping.

Doughs were mixed and remixed in the GRL mixer (15) in an atmosphere of nitrogen after evacuation of the mixing bowl. In some experiments mixing was done in oxygen. Evacuation of the bowl prior to remixing inflates a dough to several times its original volume; this inflation, however, has no significant effect on the structural relaxation. The sum of initial mixing and remixing times was always 210 seconds. It may be argued that before remixing a dough was not exactly in the same state as its control dough, since the latter obtained a longer initial mixing. Because variations in the duration of initial mixing time have only a slight effect on structural relaxation (6), similar results would have been obtained with the same initial mixing times for remixed and control doughs.

An incidental modification of the method is that two test pieces of 150 g. were scaled just prior to shaping; this reduced the number of remixing operations by a factor of two. The time needed for weighing, rounding, and shaping prevented the use of second intervals shorter than 3 minutes.

Eight test pieces were stretched after various rest periods ranging from 4 to 45 minutes for the lowest and from 7 to 120 minutes for the highest resistances. Corrected loads at 7 cm. kymograph paper displacement were read. From the scatter of the experimental points around the linear transformation of the structural relaxation curve, standard errors of estimate for the relaxation constant C and the asymptotic load L_a were calculated by means of formulas from correlation analysis (11). They were 100 to 200 g.-minutes for C, generally

increasing with C, and 4 g. for L_a. The reproducibility of C was in agreement with this standard error. Day-to-day variations in L_a, however, were larger than corresponds with this standard error.

The Halton extensigraph was used with relaxometer dough holders and accessories for loading them as described earlier (14). Three test pieces of 100 g. were scaled prior to loading the sample holders. Further, the procedure was as described above for the Brabender extensigraph. This method avoids the vigorous working of dough during rounding and shaping in the latter machine. For comparison, in some experiments the test pieces were rounded 30 times on the Brabender extensigraph rounder.

Dough temperature was maintained at 30°C. during all operations.

Results

Brabender Extensigraph — General Observations. With the Brabender extensigraph, plots of the product of load and rest period vs. rest period yielded straight lines, irrespective of whether doughs were remixed or not, independently of the second interval. Remixing does not alter the fact that the structural relaxation curve can be described as a hyperbola. Therefore the effect of remixing can be adequately recorded by changes in the structural relaxation constant C and the asymptotic load L_a. Remixing did not significantly affect the relation between the load at 7 cm. and the extensibility of doughs.

Duration of Remixing. Remixing decreases both C and L_a. Fig. 1 shows the dependence on the duration of remixing of this effect for doughs with various additions of bromate. After the longer durations of remixing, doughs were sticky and difficult to handle. On the basis

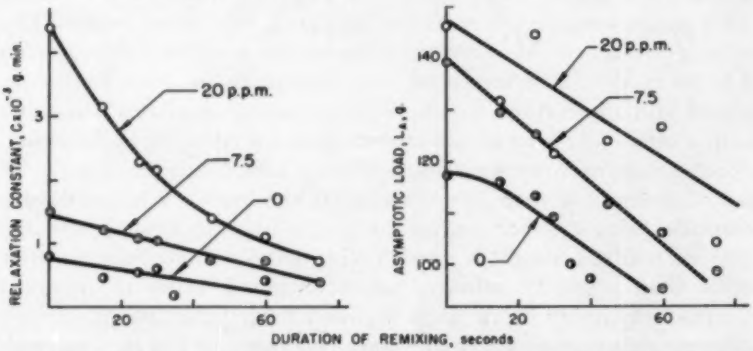

Fig. 1. Dependence of the structural relaxation constant (left) and the asymptotic load (right) on the duration of remixing for doughs without and with 7.5 and 20 p.p.m. bromate. First interval 177 minutes, second interval 3 minutes.

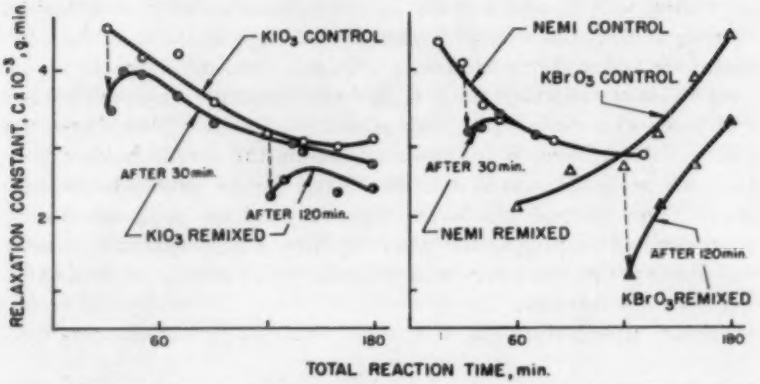

Fig. 2. Dependence of the srtuctural relaxation constant on the second interval (between remixing and shaping) for doughs containing 12.5 p.m.m. iodate (left), 20 p.p.m. bromate (right), or 35 p.p.m. N-ethylmaleimide (right). First intervals (between initial mixing and remixing) were either 30 (iodate and NEMI) or 120 minutes (iodate and bromate). Total reaction time, which is the sum of the constant first and the variable second interval, is plotted along the abscissa. Dashed arrows indicate the immediate effect of remixing.

of these results a duration of remixing of 60 seconds was chosen for all experiments reported below.

Second Interval. The influence of the length of the second interval (between remixing and shaping) was studied in a series of experiments with constant first interval (between initial mixing and remixing). Figure 2 shows how the effect of remixing on C depends upon the second interval for doughs with 12.5 p.p.m. iodate, 20 p.p.m. bromate, or 35 p.p.m. N-ethylmaleimide. The effect of remixing is largest for the shortest second interval that was practicable, that is 3 minutes. This maximum effect is indicated in Fig. 2 by dashed arrows. The effect decreases considerably when the second interval increases from 3 to 20 minutes as is shown by the approach of the lines for the remixed and the control doughs with increasing total reaction time; with a sufficiently long second interval between remixing and shaping, dough shows recovery. In the experiments with N-ethylmaleimide for second intervals of more than 20 minutes, the line for remixed doughs coincides with that for control doughs. With bromated doughs the line for remixed doughs is parallel with that for control doughs after more than about 20 minutes' second interval. Lines for remixed doughs, containing iodate, show recovery during the first 20 minutes after remixing, after which the lines run more or less parallel with that for control doughs, though at a much closer distance than with bromated doughs. This means that, whether recovery is complete or

not, it does not proceed after more than about 20 minutes after remixing. Results not reported here in detail, from experiments with constant total reaction time and various first and second intervals, support these conclusions.

Type of Improver and Reaction Time. Figure 2 showed already

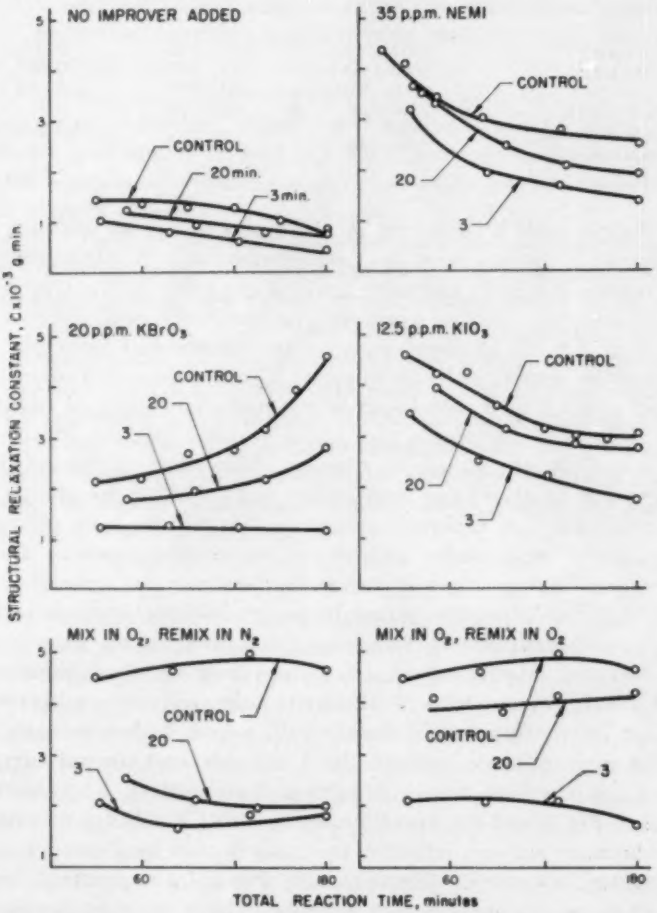

Fig. 3. Dependence of the sensitivity to remixing and of the recovery on the type of improver and reaction time. The curves for 3 minutes' second interval (between remixing and shaping) are supposed to indicate the sensitivity to remixing, and those for 20 minutes the maximum recovery. Total reaction time, which is the sum of the variable first interval (between initial mixing and remixing) and the constant second interval, is plotted along the abscissa.

TABLE I

RELATIVE VALUES OF THE STRUCTURAL RELAXATION CONSTANT C FOR REMIXED DOUGHS

IMPROVER	RANGE OF VALUES FOR C FOR CONTROL DOUGHS	C FOR REMIXED DOUGH DIVIDED BY C FOR CONTROL DOUGH AFTER THE SAME TOTAL REACTION TIME	
		3 Minutes' 2nd Interval	20 Minutes' 2nd Interval
	g/minute		
NEMI, 35 p.p.m.	2500–3800	0.55–0.85	0.75–1.00
KIO_3, 12.5 p.p.m.	3000–4300	0.55–0.75	0.85–0.90
None	750–1400	0.45–0.70	0.70–0.90
$KBrO_3$, 20 p.p.m.	2200–4500	0.25–0.60	0.60–0.80
O_2 (remixing in O_2)	4400–5000	0.30–0.45	0.80–0.90
O_2 (remixing in N_2)			0.40–0.55

that doughs with bromate are much more sensitive to remixing and recover to a lesser extent than do doughs with N-ethylmaleimide. The results of a more extensive study of the effect of the type of improver and the reaction time on the sensitivity and recovery are shown in Fig. 3. In these experiments various first intervals were followed by second intervals of either 3 or 20 minutes. Curves for a second interval of 3 minutes show the maximum effect of remixing. On the basis of the results above it is assumed that the 20 minutes' curves indicate the maximum recovery. The course of control curves in Fig. 3 is in agreement with earlier work (5,7,8); the effect of N-ethylmaleimide on structural relaxation has not yet been published. The control experiments with N-ethylmaleimide, bromate, and iodate cover similar ranges of values for the structural relaxation constant C. However, doughs mixed in oxygen had slightly higher values for C, and doughs without improvers much lower values. To overcome this difficulty, relative values of C as read from Fig. 3 are summarized in Table I. Doughs with N-ethylmaleimide are least sensitive to remixing, closely followed by doughs with iodate. This is demonstrated by the short distance between the 3 minutes' and control curves in Fig. 3 and the high figures in column 3 of Table I. The larger distance in Fig. 3 and the lower figures in Table I indicate that doughs with bromate are very sensitive, particularly after long reaction times; this is the only case in which reaction time has a large effect. Doughs mixed in oxygen are also very sensitive. The position of the 20 minutes' curves in Fig. 3 and the figures in column 4 of Table I show that after recovery the rank order of doughs with different improvers is essentially the same as after a second interval of only 3 minutes. Recovery is even complete for doughs with N-ethylmaleimide after re-

action times of less than 60 minutes. Doughs mixed in oxygen and remixed in nitrogen hardly show any recovery; after remixing in oxygen, however, recovery is considerable.

Dough with Both Iodate and Bromate. The behavior of doughs with both 6.25 p.p.m. iodate and 10 p.p.m. bromate is intermediate between that of doughs with either 12.5 p.p.m. iodate or 20 p.p.m bromate alone. After short reaction times the behavior is more like that of doughs with iodate, and after long reaction times more like that of bromated doughs.

Asymptotic Load. The asymptotic load L_a generally follows the behavior of the structural relaxation constant C but in a less consistent way as is illustrated in Fig. 1. L_a of iodated doughs is not significantly affected by remixing. In agreement with earlier work (7,8), a large effect of oxygen on L_a was found.

Halton Extensigraph. Structural relaxation curves obtained with the Halton extensigraph for doughs with 20 p.p.m. bromate and after 180 minutes' reaction time showed a much larger scatter of experimental points than did curves obtained with the Brabender extensigraph. Moreover, they did not fit the usual equations for a hyperbola. However, qualitative information on the interaction of remixing and structural activation could be obtained. Rounding of the test pieces raises the structural relaxation curve, presumably by structural activation, and remixing with a 4 minutes' second interval lowers them. The effect of structural activation is much more marked in control doughs than if it follows remixing.

Discussion

The present experiments confirm the observation of Freilich and Frey (12) that remixing after fermentation is a means of canceling the effect of excess bromate. Figure 1 illustrates that remixing reduces the structural relaxation constant C and the asymptotic load L_a to values that correspond with a lower bromate level. Freilich and Frey, by using long fermentation time, adopted good conditions for a demonstration of the effect of remixing; according to the present experiments, doughs with bromate after a long reaction time are most sensitive to remixing.

Two observations can easily be understood. The first is the difference between doughs initially mixed in oxygen, and remixed in nitrogen and in oxygen, respectively, followed by a second interval of 20 minutes between remixing and shaping. Remixing in nitrogen reduces the duration of the improvement reaction but not of degradative processes. Remixing in oxygen brings the improver concentra-

tion up again to its original level, thus favoring the improvement reaction. The second observation is the behavior of doughs with both 6.25 p.p.m. iodate and 10 p.p.m. bromate, which is in agreement with the fast reaction of iodate and the slow reaction of bromate (5).

Recovery attains its maximum about 20 minutes after remixing. Of course the validity of this conclusion is confined to unleavened doughs from the present flour with the present water content and at 30°C. It is in remarkably good agreement with the recovery times of 25 minutes used in test baking (1) and 15–20 minutes in practice (2).

The decrease in extensigram height caused by remixing illustrates a decrease in rigidity of dough. Assuming that the rigidity is due to crosslinks between peptide chains, it is logical to suppose that remixing breaks some of the crosslinks, and that new crosslinks are formed during the first 20 minutes of the second interval. Since the energy applied to the dough during remixing is in the order of only 10 gcal. per $cm.^3$, or about 13 kcal. per mole amino acid residue, part of which is converted into heat, breaking of primary chemical bonds cannot occur to a large extent. Chain entanglements could be the crosslinks disappearing by remixing. During rest they may be re-formed as a consequence of Brownian motion. This explanation is related to those usually given for thixotropy and structural viscosity.

In the present experiments there are two operations that affect the rigidity of dough. Rounding and shaping cause an increase in the rigidity, which is referred to as structural activation (7); presumably this is due to the formation of crosslinks to an extent that depends upon chemical reactions in the preceding reaction time. The second operation is remixing, which decreases the rigidity. These operations are followed by the processes structural relaxation and recovery, during which their effects gradually decrease. Results of the present experiments indicate that structural activation, followed by relaxation, and breakdown, due to remixing, and followed by recovery, do not proceed independently. The experiments with the Halton extensigraph demonstrate that remixing has a smaller effect if structural activation is minimized. This fact finds its counterpart in the observation that the effect of remixing is largest in bromated doughs after a long reaction time; previous investigations (5,7-10), including work with bromated doughs in the Chopin extensigraph (3,16), has shown that under these conditions structural activation is more obvious than in any other of the present experiments. Moreover, in some cases the effect of remixing could not be observed by palpating doughs before rounding and shaping, whereas extensigrams, obtained after shaping, showed it clearly. Finally, there is no indication that the progress of

recovery in the course of time obeys an equation related to the hyperbolic equation for structural relaxation. Therefore, one cannot expect that structural relaxation curves after remixing and second intervals shorter than 20 minutes, could be described as hyperbolas if breakdown, due to remixing, and recovery were merely superposed on structural activation and relaxation. The fact that all experimental data could very well be described by the hyperbolic equation also suggests that structural activation was affected by previous remixing. It cannot be decided on the basis of the present experiments whether a complete omission of structural activation would result in no effect of remixing. The hypothesis that remixing interferes with subsequent structural activation is not necessarily contradictory to the assumption made above, that the effect of remixing is due to chain disentanglement. It is possible that the work of rounding and shaping results in structural activation only if there is a sufficient number of chain entanglements.

The large differences in sensitivity to remixing and in recovery between doughs with different improvers demonstrate that the improvers act in different ways. The addition of N-ethylmaleimide or iodate results in doughs with little sensitivity and good recovery, whereas bromate and oxygen are associated with high sensitivity and poor recovery. Remarkably, the dividing line between these two groups of flour improvers does not coincide with the distinction between oxidizing and thiol-blocking reagents. The behavior of the oxidizing potassium iodate is very similar to that of the only thiol-blocking reagent used in these experiments, N-ethylmaleimide. This is another argument in favor of the idea that flour improvement is due to disappearance of thiol groups rather than to the formation of disulfide cross-links (4,13). Although the position of oxygen in this respect is not completely clear, the dividing line between improvers inducing high and low sensitivity to remixing seems to be correlated with the slope of the plot of C vs. reaction time, that is, with the rate of change of rheological properties induced by them. Rapidly acting flour improvers seem to result in slightly sensitive doughs, whereas slowly acting reagents lead to highly sensitive doughs. The poor recovery of bromated doughs cannot be due to the presence of residual bromate, since then doughs with both iodate and bromate would also have shown poor recovery after short reaction times.

Literature Cited

1. Anderson, J. A. Grain Research Laboratory 1958 Report, p. 6.
2. Anonymous. Full-flavored bread from Patterson's new process. Baker's Digest 33 (3): 48–49 (1959).

3. BENNETT, RUTH, and COPPOCK, J. B. M. Measuring the physical characteristics of flour: a method of using the Chopin Alveograph to detect the effect of flour improvers. J. Sci. Food Agr. **3:** 297–307 (1952).
4. BLOKSMA, A. H. Die Bedeutung von Thiol- und Disulfidgruppen im Kleber für die Backfähigkeit. Getreide u. Mehl **8:** 65–69 (1958).
5. DEMPSTER, C. J., CUNNINGHAM, D. K., FISHER, M. H., HLYNKA, I., and ANDERSON, J. A. Comparative study of the improving action of bromate and iodate by baking data, rheological measurements, and chemical analyses. Cereal Chem. **33:** 221–239 (1956).
6. DEMPSTER, C. J., and HLYNKA, I. Some effects of the mixing process on the physical properties of dough. Cereal Chem. **35:** 483–488 (1958).
7. DEMPSTER, C. J., HLYNKA, I., and ANDERSON, J. A. Extensograph studies of structural relaxation in bromated and unbromated doughs mixed in nitrogen. Cereal Chem. **30:** 492–503 (1953).
8. DEMPSTER, C. J., HLYNKA, I., and ANDERSON, J. A. Extensograph studies of the improving action of oxygen in dough. Cereal Chem. **31:** 240–249 (1954).
9. DEMPSTER, C. J., HLYNKA, I., and ANDERSON, J. A. Influence of temperature on structural relaxation in bromated and unbromated doughs mixed in nitrogen. Cereal Chem. **32:** 241–254 (1955).
10. DEMPSTER, C. J., HLYNKA, I., and WINKLER, C. A. Quantitative extensograph studies of relaxation of internal stresses in non-fermenting bromated and unbromated doughs. Cereal Chem. **29:** 39–53 (1952).
11. EZEKIEL, M. Methods of correlation analysis, chap. 18. Wiley: New York (1930).
12. FREILICH, J., and FREY, C. N. Dough oxidation and mixing studies. II. Effects of remixing after fermentation. Cereal Chem. **16:** 495–502 (1939).
13. GOLDSTEIN, S. Sulfhydryl- und Disulfidgruppen der Klebereiweisse und ihre Beziehung zur Backfähigkeit der Brotmehle. Mitt. Gebiete Lebensm. u. Hyg. (Bern) **48:** 87–93 (1957).
14. HLYNKA, I. Some observations on the "research" extensometer. J. Sci. Food Agr. **6:** 763–767 (1955).
15. HLYNKA, I., and ANDERSON, J. A. Laboratory dough mixer with an air-tight bowl. Cereal Chem. **32:** 83–87 (1955).
16. HLYNKA, I., and BARTH, F. W. Chopin alveograph studies. II. Structural relaxation in dough. Cereal Chem. **32:** 472–480 (1955).

EFFECT OF MALTING PROCEDURE AND WHEAT STORAGE CONDITIONS ON ALPHA-AMYLASE AND PROTEASE ACTIVITIES[1]

JAMES R. FLEMING, JOHN A. JOHNSON, AND BYRON S. MILLER[2]

ABSTRACT

Factors influencing alpha-amylase and protease activities of wheat malted on a laboratory scale were studied. Wheat malts were produced by steeping to 40–46% moisture and germinating at 42° to 68°F. for 1 to 12 days. Germination time was the most important factor influencing enzyme production. The optimum malting conditions appeared to be approximately 42% steep moisture level and 60°F. germination temperature. Kilning temperatures above 110°F. caused loss of both enzymes.

Enzyme activities of freshly harvested wheat did not reach their maximum until after 2 months of storage. Storage at low temperatures (40°F.) favored production of greater alpha-amylase and protease activities.

Numerous studies of the effects of malting conditions on the production of alpha-amylase have been made and the conclusions reached have agreed quite well. Geddes, Hildebrand, and Anderson (5), Kneen, Miller, and Sandstedt (6), Dickson, Olson, and Shands (3), and Dickson and Shands (2) demonstrated that increased steeping moisture, germination time, and germination temperature increased alpha-amylase production.

Relatively little information, however, is available concerning the effect of malting conditions on protease production. It is generally believed that protease activity parallels the alpha-amylase activity of germinated cereals. Geddes *et al.* (5) reported that the autolytic protease activity of malted wheat flour was augmented by longer periods of germination, increased steep levels, and higher protein content. Otis and Olson (10) demonstrated that proteolytic activity of barley malts was enhanced when the steep moisture was increased, but the duration of the germination period was more important than the germination moisture. Mounfield (9) reported that wheats which were stored for 2 years at 18°C. lost much of their ability to produce proteases during germination.

The primary purpose of this investigation was to obtain information concerning the factors influencing protease and alpha-amylase

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production during the malting of wheat. Several hundred malts were produced by employing a wide range of steep moisture levels, germination times, germination temperatures, kilning temperatures and wheat storage conditions. Procedures such as programmed kilning, in which temperatures are increased as the moisture content of the malt is lowered, were not employed in order to simplify operations.

Materials and Methods

Malting Equipment. The design of the laboratory malting equipment was evolved through visits to several wheat-malting laboratories. The steeping, germinating, and kilning of wheats were carried out in parabolic-shaped wire screen baskets holding 1.5 lb. of malted wheat. The steeping bath was an insulated, refrigerated, stainless-steel tank. It was provided with a water circulation pump and intake and overflow pipes to assure continual aeration and exchange of water. The temperature was maintained thermostatically within ±1°F. The germinator was a large commercial refrigerator which was equipped with additional cooling and heating coils and air-circulating and humidifying units. The desired temperature and a saturated atmosphere were maintained by means of thermostatic controls and a Minneapolis-Honeywell temperature and humidity recorder. The wire baskets containing the germinating grain were held by means of a revolving arm assembly which was rotated at 5 revolutions per hour. In this manner the grain was slowly moved to provide for proper aeration and to guard against overheating of portions of the sample. The sprouted wheats were dried in a large forced-air convection-type oven which was equipped with a revolving assembly to facilitate uniform drying of the green malts.

Malting Procedure. The standard wheat was a sample of Pawnee grown at Manhattan, Kansas. One-pound lots were routinely used for the production of each malt. All lots were steeped at 50°F. for the time necessary to bring the moisture content to the 40, 42, 44, and 46% levels. Germination was carried out at 42°, 50°, 60°, and 68°F. for 1 to 12 days. All of the green malts, except those employed in the kilning temperature study, were dried at 104°F. for 24 hours. Rootlets and acrospires were removed by kneading the dried malts in a plastic bag.

In the study of the effects of drying temperatures on enzyme activities, the wheats were steeped to 42% moisture content and germinated at 68°F. for 6 days. Drying temperatures ranged from 100° to 150°F.

To check the precision of the malting equipment and procedures,

a number of portions of wheat were malted for 2, 4, 6, and 8 days at 68°F. The malts were assayed for alpha-amylase and protease activities after drying and cleaning.

To study the effect of the age of wheat and storage temperatures, two samples of hard red winter wheats (Concho and Triumph) each were divided into three lots immediately after harvest. One lot of each wheat was stored at 40°F., 70° to 80°F. (room temperature) and 100°F. One-pound samples of each lot were malted after 1, 2, 4, 6, and 7 months of storage. Germination was carried out at 42% moisture and 68°F. for 8 days. Malts were dried at 104°F. for 24 hours.

Analytical Methods. The rate of water absorption for each variety of wheat was determined by a modification of the method of Banasik *et al.* (1). Twenty-five grams of wheat were steeped in perforated polyethylene bags for 16 to 96 hours. Samples were transferred to tared, perforated, plastic centrifuge tubes and centrifuged for 5 minutes at 1,800 r.p.m. The gain in weight was then used to calculate the steep moisture level attained. When steeping was performed in perforated plastic centrifuge tubes in accordance with the original procedure (1) used for barley, the swelling of the wheat mass was restricted and abnormal water absorption values were obtained.

Alpha-amylase activity was determined by the method of Sandstedt, Kneen, and Blish (12) as modified by Redfern (11). Protease activities were determined by the method of Miller and Johnson (8) and the gelatin viscosity technique of Koch and Ferrari (7). Malting losses were determined by comparing the dry weights of 1,000 kernels of the original and malted wheats. All of the enzyme activities are those found in the finished malt and expressed on a dry matter basis.

Results and Discussion

Data concerning the replicability of the malting procedure are given in Table I. The small standard deviation of each set of replicates established the dependability of the malting procedures and equipment.

The two procedures used to determine protease activities were highly correlated ($r = 0.993$, 31 d.f.). It was decided, therefore, to use the Miller-Johnson method (8) for routine protease determinations.

Effect of Germination Temperature. Enzyme production (Fig. 1, graphs A and C) was sharply curtailed in malts germinated at 42°F. or 50°F. compared with those germinated at 60°F. or above. Relatively small increases in activity were noted when the temperature was raised from 60° to 68°F. For further routine work, 60°F. was selected for

TABLE I

REPLICABILITY OF THE MALTING PROCEDURE AS MEASURED BY ALPHA-AMYLASE ACTIVITY, PROTEASE ACTIVITY, AND MALT YIELD[a]

Replication No.	Germination Time (Days)								
	2	4	8	2	4	8	2	4	8
	Alpha-Amylase Activity			Protease Activity			Malt Yield		
	SKB units/g	*SKB units/g*	*SKB units/g*	*HU/g*	*HU/g*	*HU/g*	%	%	%
1		209	363		84.1	101.4		92.3	87.6
2		218	378		87.5	98.7		91.7	86.4
3	84	200	361	61.5	81.9	102.0	96.5	92.5	87.1
4	65	202	354	62.8	84.5	106.4	96.1	91.8	85.9
5	79	219	374	59.7	84.9	101.7	97.2	91.3	87.3
6	80	209	360	60.7	84.1	102.5	96.7	92.3	86.6
Mean	77	210	365	61.2	84.5	102.2	96.6	92.0	86.8
Std. Dev.	8.3	7.8	9.1	1.3	1.8	2.4	0.70	0.46	0.63

[a] Conditions: Pawnee wheat steeped to 42% moisture, germinated at 68°F., and kilned at 104°F. for 24 hours.

the germination temperature.

Effect of Steep Moisture. Figure 1, graphs B and D, shows that enzyme activities were increased with each increment in the steep moisture content. Additional data (unpublished), obtained with lower steep levels, indicated that 40% was the lowest practical level for acceptable response. Singruen (13) stated that the moisture content should be optimal at the initiation of germination, since modification of other conditions would not compensate for inadequate moisture. The indicated preference for steep moisture levels in the 40–42% range confirms the earlier work of Geddes *et al.* (5) and Dickson and Geddes (4). Steep moisture levels higher than 40–42% increased the enzyme production only slightly. For further routine work a steep moisture level of 42% was adopted.

Effect of Germination Temperature and Steep Moisture Level on Malt Yield. The effect of germination temperature and time on malt yield is shown in Fig. 2, A. Increasing germination temperatures not only decreased the malt yield but created a mold growth problem at the highest temperature for certain wheat samples. Germination time ranging from 1 to 12 days decreased the malt yield approximately 10%.

Increasing the steep moisture level (Fig. 2, B) decreased the malting yield. When steep moisture level was increased from 40 to 46%, the steep time was increased 20–30 hours, depending on the sample of wheat. Decrease in yield during 12 days of germination amounted to approximately 10% depending on the steep moisture level. The decrease during steeping, germination, and drying reflected losses due to leaching, respiration, and root and shoot growth.

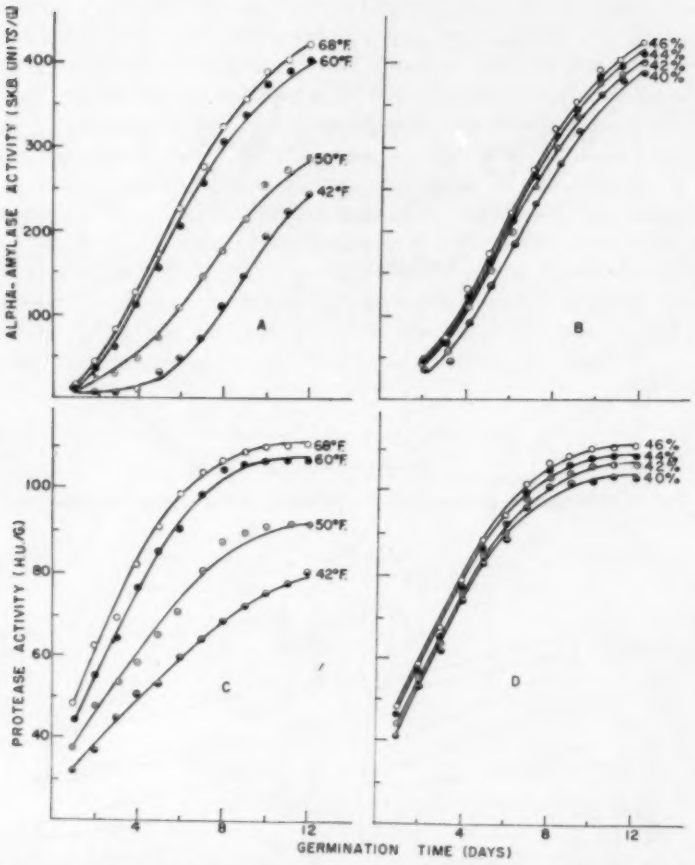

Fig. 1. Influence of germination temperature and steeping moisture level on alpha-amylase and protease activities of malted wheat.

Effect of Kilning Temperature. The influence of drying temperatures applied throughout the whole period of kilning on enzyme activities is shown in Fig. 3. A gradual decrease took place over the full range of the temperatures studied. The lowest practicable temperature that could be maintained under the conditions of these experiments was 104°F. While gross differences in both protease and alpha-amylase activities are reflected in the different varieties and classes of wheat, the effect of drying temperature, except for the protease of Pawnee, is essentially uniform.

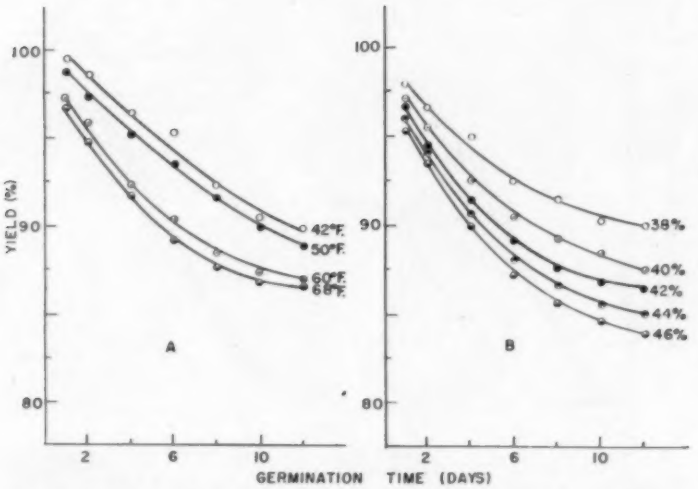

Fig. 2. Influence of germinating temperature and steeping moisture level on malt yield.

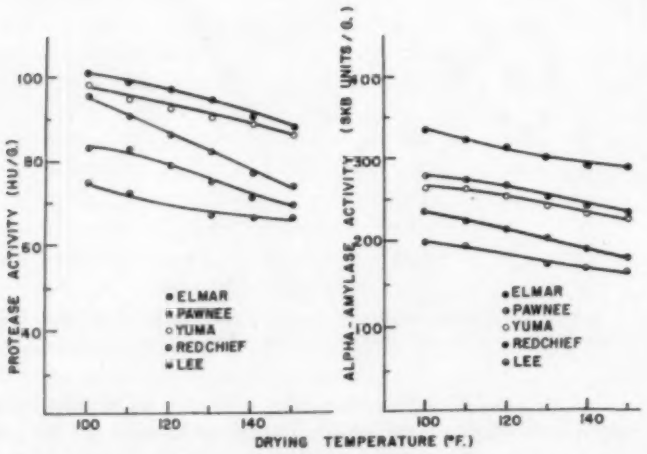

Fig. 3. Influence of drying temperature on alpha-amylase and protease activities of malted wheat of various varieties.

Witt (14) demonstrated that malt enzymes were more susceptible to heat when the moisture content of the malt was high. Since only one temperature was used in the routine kilning of the present samples, the data may not be interpretable to commercial malting opera-

tions which generally employ higher kilning temperatures after most of the moisture has been removed at lower temperatures.

Effect of Storage Temperature and Time on Malting Properties of Wheats. The effect of wheat storage at different temperatures is shown in Fig. 4. The freshly harvested wheats germinated poorly because of the temporary dormancy of the seeds at this stage of maturation. The percentage of seeds that germinated, the acrospire lengths, and the alpha-amylase and protease activities all increased appreciably during the first 2 months of storage. The enzyme activities of wheats stored at 80° or 100°F. did not reach the levels of those stored at 40°F. Viability of seeds stored at 100°F. was reduced during the storage period, but not to the same extent as was the production of alpha-amylase and protease enzymes. The data thus indicate that the con-

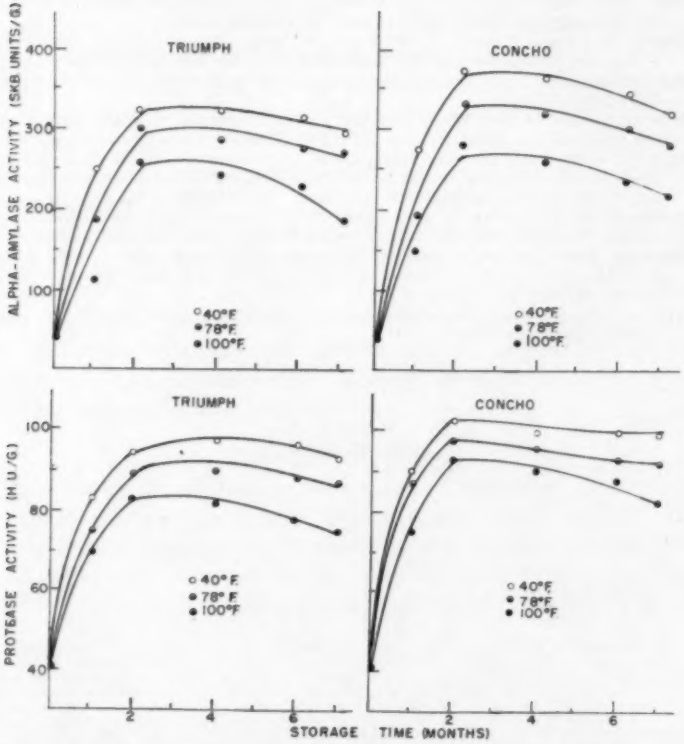

Fig. 4. Influence of wheat storage conditions on alpha-amylase and protease activities of malted wheat of two varieties.

ditions under which wheat is stored prior to malting are extremely important.

Literature Cited

1. BANASIK, O. J., MYHRE, D., and HARRIS, R. H. A micro-malting method for nursery samples. Brewer's Dig. **31:** 50–55 (1956).
2. DICKSON, A. D., OLSON, W. J., and SHANDS, H. L. Amylase activity of three barley varieties as influenced by different malting conditions. Cereal Chem. **24:** 325–337 (1947).
3. DICKSON, A. D., and SHANDS, H. L. The influence of the drying procedure on malt composition. Cereal Chem. **19:** 411–419 (1942).
4. DICKSON, J. G., and GEDDES, W. F. Effect of wheat class and germination moisture and time on the malt yield and amylase activity of malted wheat. Cereal Chem. **26:** 404–414 (1949).
5. GEDDES, W. F., HILDEBRAND, F. C., and ANDERSON, J. A. The effect of wheat type, protein content, and malting conditions on the properties of malted wheat flour. Cereal Chem. **18:** 42–60 (1941).
6. KNEEN, E., MILLER, B. S., and SANDSTEDT, R. M. The influence of temperature on the development of amylase in germinating wheat. Cereal Chem. **19:** 11–27 (1942).
7. KOCH, R. B., and FERRARI, C. G. Investigation of proteolytic enzymes by a gelatin viscosity technic. Cereal Chem. **32:** 254–269 (1955).
8. MILLER, B. S., and JOHNSON, J. A. A simple linear relationship and definition of a unit for proteinase activity. Arch. Biochem. **32:** 200–206 (1951).
9. MOUNFIELD, J. D. The proteolytic enzymes of sprouted wheat. II. Biochem. J. **30:** 1778–1786 (1936).
10. OTIS, O. J., and OLSON, W. J. The effect of germination moisture and time on malt proteolytic activity. Proc. Am. Soc. Brewing Chemists **1953**, pp. 22–25.
11. REDFERN, S. Methods for determination of alpha-amylase. IV. A glass end-point color standard for use in the dextrinizing method: effect of temperature and starch lot on this method. Cereal Chem. **24:** 259–268 (1947.)
12. SANDSTEDT, R. M., KNEEN, E., and BLISH, M. J. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16:** 712–723 (1939).
13. SINGRUEN, ELSIE. Review of brewing literature issued from June 1, 1935 to June 1, 1936, with special reference to barley, malt, and malt adjuncts. Cereal Chem. **14:** 410–418 (1937).
14. WITT, P. R., JR. Effect of kilning on the amylolytic activity of barley malts. Cereal Chem. **22:** 341–349 (1945).

EFFECT OF ENVIRONMENT, VARIETY, AND CLASS OF WHEAT ON ALPHA-AMYLASE AND PROTEASE ACTIVITIES OF MALTED WHEAT[1]

JAMES R. FLEMING, JOHN A. JOHNSON, AND BYRON S. MILLER[2]

ABSTRACT

The effects of environment, wheat class, and variety on alpha-amylase and protease activities of malted wheat have been investigated. Because of the large variety-station interaction, the effect of variety and station on enzyme activities of hard red winter wheat malts was nonsignificant. Within a given station or variety significant differences were observed. On the basis of the enzyme activities of their malts, the wheat classes were rated in the following order: soft white, soft red winter, hard red winter, durum, and hard red spring.

The barley malting industry has recognized, through its routine surveys, that varieties differ appreciably in their malting properties. Dickson and Geddes (6), Anderson and co-workers (1,2,12) and Kneen and Hads (11) have demonstrated that classes and varieties of barley and wheat vary in their ability to produce satisfactory malts. Dickson and Geddes (6) and Geddes *et al.* (9) rated soft white wheat as the best for malting, followed by soft red winter, hard red winter, and hard red spring wheats.

Kneen and Hads (11) showed that alpha- and beta-amylase activities of malted wheats depended on environment as well as variety. Meredith *et al.* (12), Dickson *et al.* (5,7), and Banasik and Harris (3,10) have also demonstrated the importance of station and crop year. These earlier studies were concerned with varieties now of minor importance. This investigation was designed to evaluate for malting purposes current commercial varieties.

Materials and Methods

The laboratory equipment used has been described (8). Two samples each of several varieties of the soft white winter, soft red winter, durum, hard red winter, and hard red spring classes were used for the study of varietal factors. Portions of each wheat were steeped to 40 and 42% moisture levels and germinated for periods of 2, 4, 6, 8, 10,

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and 12 days at 50°, 56°, 62°, and 68°F. Malts were kilned at 104° F. for 24 hours.

Samples of four varieties of hard red winter wheat from the 1956 crop grown at nine stations in Kansas, Langdon durum wheat grown at three locations in North Dakota, and three varieties of soft red winter wheat grown at two locations in Ohio were used for the study of the effect of environment. The hard red winter wheats were steeped to 42% moisture, germinated at 62°F. for 6 days, and dried at 104°F. for 24 hours. The full range of conditions outlined in the foregoing paragraph was employed in preparing the durum wheat malts. The soft winter wheat samples were steeped to 42% moisture, germinated at 68°F. for 12 days, and dried at 104°F. for 24 hours.

The rate of water absorption during steeping was determined by the modified method of Banasik *et al.* (4,8). Alpha-amylase was determined by the Sandstedt-Kneen-Blish method (15), as modified by Redfern (14). Protease activity was measured by the Miller and Johnson procedure (13).

Results and Discussion

Effect of Variety and Class. The alpha-amylase activities of the malts of the various classes and varieties of wheats studied are shown in Fig. 1. These data are typical for other steep-moisture levels and germination temperatures for the given varieties. The soft white wheats (Elmar and Genesee) produced malts having slightly greater activities than the soft red winter wheats. Seneca malts were appreciably lower in alpha-amylase than other varieties of its class tested. Concho was superior to others of the hard red winter class, while RedChief malts possessed the least alpha-amylase activity. Pawnee, Ponca, and Triumph were intermediate in this group, differing very slightly among themselves. The range of alpha-amylase activities of the durum wheat malts tested was not as great as that of the other classes. Among the varieties of hard red spring wheats studied, Selkirk produced the highest alpha-amylase activity, while Lee, Rescue, and Thatcher were significantly lower.

Protease activities for the same wheat malts are presented in Fig. 2. Differences in protease activities of the soft red winter wheats were slight. The hard red winter wheats exhibited a greater variation in protease activity than did the soft wheats. Concho and Pawnee, followed closely by Ponca, possessed the most protease activity, RedChief showed the least activity, while that of Triumph was only slightly greater. The protease activities of the malted hard red spring wheats were extremely variable. Rescue and Thatcher, which developed lower

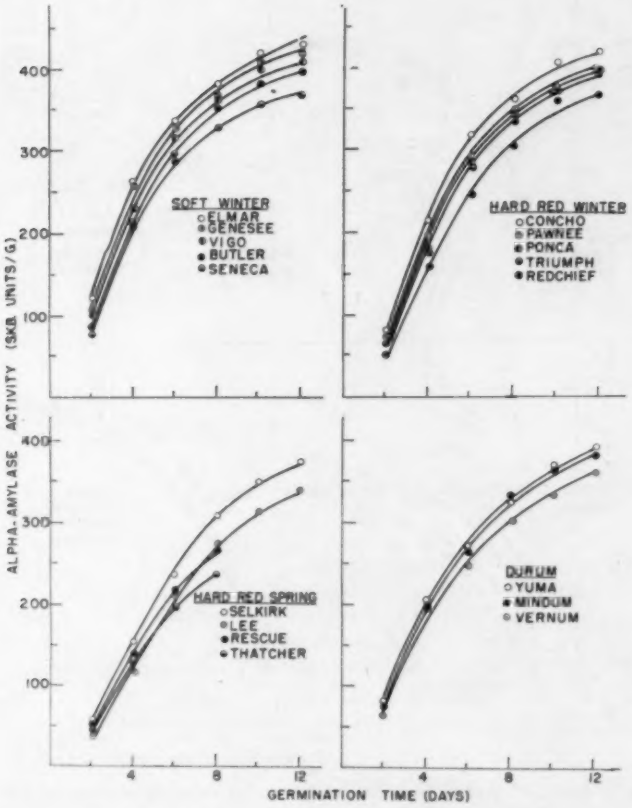

Fig. 1. Effect of variety and class of wheat on alpha-amylase activity of the malted grain.

amounts of alpha-amylase, had the highest protease activity. Lee had the lowest protease activity of all the hard red spring wheats tested. In the durum class, Mindum was higher in protease than Yuma and Vernum.

The average protease and alpha-amylase activities for the various wheat classes are given in Fig. 3. The soft white wheats were superior as a class to all others. They were not only pre-eminent to the others when malted under the given conditions, but also excelled when germinated at lower temperatures (Table I). Genesee wheat malted at 50°F. for 4 days had enzyme activities approximately equivalent to those of Concho wheat germinated for 6 days. Eight-day Genesee malt

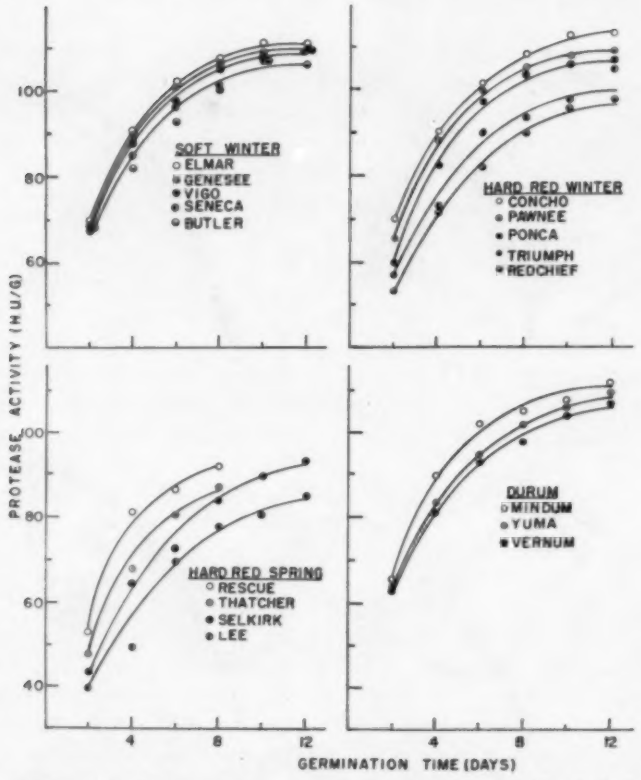

Fig. 2. Effect of variety and class of wheat on protease activity of the malted grain.

had enzyme activities equal to those of Concho germinated for 10 to 12 days.

The average protease and alpha-amylase activities of the soft red winter class were less than those of the soft white wheats (Fig. 3). The differences between these classes, however, were slight compared with those between the soft and hard red spring wheats. The hard red winter wheat class was superior to the hard red spring wheats in alpha-amylase and protease activity. The enzyme activities of the durums were similar to those for the hard red winter wheats. These data agree with those of Dickson and Geddes (6).

Effect of Variety and Environment. A study of the effect of environment and variety on the enzyme activities of hard red winter wheats is indicated by the data in Tables II and III. There were no

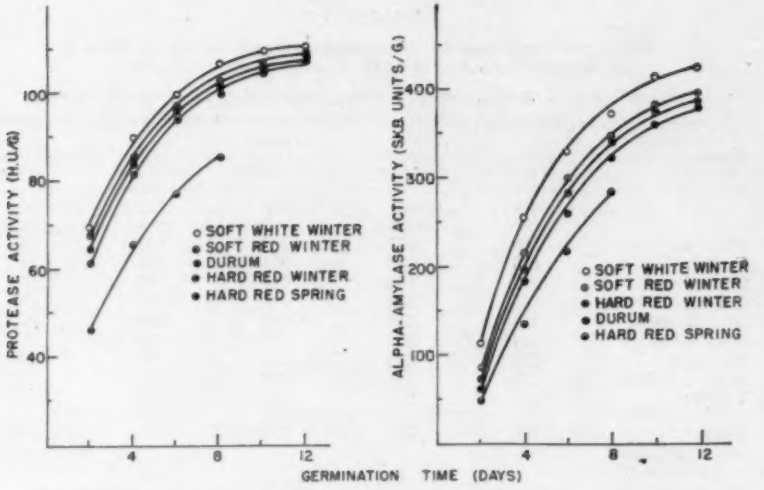

Fig. 3. Effect of class of wheat on alpha-amylase and protease activities of the malted grain.

TABLE I

ALPHA-AMYLASE AND PROTEASE ACTIVITIES OF CONCHO AND GENESEE WHEAT MALTS

(Steep moisture 42%)

VARIETY	GERMINATION TEMP.	PROTEIN	GERMINATION PERIOD (DAYS)					
			2	4	6	8	10	12
	°F	%						
			Protease (HU/g of malt)					
Concho[a]	50	12.7	40.3	54.7	68.5	80.0	87.8	91.5
Genesee[b]	50	10.4	49.7	66.0	80.6	90.1	95.6	99.6
Concho	62	12.7	56.4	77.0	87.8	96.7	102.2	107.1
Genesee	62	10.4	66.3	80.8	94.3	102.0	105.9	108.9
			Alpha-amylase (SKB units/g of malt)					
Concho	50	12.7	27	52	106	176	240	288
Genesee	50	10.4	46	105	182	239	291	316
Concho	62	12.7	79	203	297	358	381	424
Genesee	62	10.4	103	243	314	360	402	422

[a] Hard red winter.
[b] Soft white winter.

significant variety or station differences compared to within-variety and station variance for either alpha-amylase or protease activities. However, within a given station or variety, significant differences were observed in many cases.

TABLE II

EFFECT OF ENVIRONMENT AND VARIETY ON THE ALPHA-AMYLASE OF MALTED HARD RED WINTER WHEATS OF THE 1956 CROP

(Wheat steeped to 42% moisture and germinated at 62° F. for 6 days)

STATION	VARIETY				
	Ponca	Pawnee	Triumph	RedChief	Average
	SKB units/g	*SKB units/g*	*SKB units/g*	*SKB units/g*	*SKB units/g*
Belleville	215	241	207	198	217
Canton	196	238	214	195	211
Columbus	217	190	209	183	200
Garden City	182	208	183	183	189
Hutchinson	235	239	219	200	223
Manhattan	214	224	217	213	217
Mankato	222	209	202	197	208
Mound Valley	205	224	196	206	208
Thayer	197	205	193	177	193
Mean	209	219	204	195	
Least significant difference = 10					

ANALYSIS OF VARIANCE

Source of Variation	D.F.	Mean Square
Variety	3	3353
Station	8	1735
Variety × station	24	396 ***
Replications, same station	9	1013 ***
Replications × variety, same stations	27	43.9 ns
Remainder	72	13.3

Pawnee tended to be highest in alpha-amylase, being equivalent or superior to Ponca at all stations except Columbus and Mankato. Ponca generally was ranked second to Pawnee. Triumph was third in this group in alpha-amylase, while RedChief was never significantly higher than any other variety at any station. The wheats grown at Belleville, Hutchinson, and Manhattan, followed rather closely by those grown at Mankato, Mound Valley, and Canton, produced malts having the highest alpha-amylase activities. The malts of wheats produced at Garden City and Thayer were significantly lower in alpha-amylase than those from other stations.

The protease activities of all Pawnee and Ponca malt samples were essentially equal for most of the stations. Triumph ranked third in protease activity, being higher than RedChief in all cases, except for the Garden City-grown samples. The samples from Belleville, Hutchinson, and Canton gave malts with the highest protease activity, while those from Garden City, Thayer, Columbus, and Mound Valley were least active.

TABLE III

EFFECT OF ENVIRONMENT AND VARIETY ON THE PROTEASE ACTIVITY OF MALTED HARD RED WINTER WHEATS OF THE 1956 CROP

(Wheat steeped to 42% moisture content and germinated at 62°F. for 6 days)

STATION	VARIETY				
	Ponca	Pawnee	Triumph	RedChief	Average
	HU/g	*HU/g*	*HU/g*	*HU/g*	*HU/g*
Belleville	86.1	86.5	78.8	69.9	80.3
Canton	84.1	84.8	81.5	74.1	81.1
Columbus	77.1	76.0	72.3	70.5	73.7
Garden City	78.4	79.7	68.0	71.0	74.3
Hutchinson	86.7	86.7	78.7	70.0	80.5
Manhattan	82.8	78.5	75.6	70.2	76.8
Mankato	78.6	81.8	75.8	75.4	77.7
Mound Valley	75.0	75.5	71.9	67.3	72.4
Thayer	79.7	79.4	70.5	65.5	73.8
Mean	80.9	81.0	74.8	70.3	
Least significant difference = 1.9					

ANALYSIS OF VARIANCE

Source of Variation	D.F.	Mean Square	
Variety	3	809	
Station	8	148	
Variety × station	24	22	•••
Replications, same station	9	24	•••
Replications × variety, same stations	27	1.7	ns
Remainder	72	0.36	

TABLE IV

EFFECT OF ENVIRONMENT ON ENZYME ACTIVITIES OF DURUM WHEAT MALTS (VARIETY LANGDON)

(Wheat steeped to 42% moisture and germinated at 68°F. for 12 days)

STATION	PROTEIN	ALPHA-AMYLASE	PROTEASE
	%	*SKB units/g*	*HU/g*
Edgeley	12.5	414	112.3
Williston	12.2	395	110.2
Minot	12.9	379	108.9

The effect of environment on alpha-amylase and protease activities of durum wheats (variety Langdon) is shown in Table IV. The malts produced from the wheats grown at Edgeley were higher in enzyme activities than those from wheats grown at Williston and Minot. The activities of the Williston sample were slightly higher than those of the Minot-grown wheat. Banasik and Harris (3) reported that malts from barley grown at Edgeley had higher alpha-amylase activities than those produced from barleys grown at Williston or Minot.

The effect of environment on alpha-amylase and protease activities of malted soft winter wheats is given in Table V. All samples grown at Wooster produced malts with slightly higher enzyme activities than those from Columbus.

TABLE V

EFFECT OF ENVIRONMENT AND VARIETY ON THE ALPHA-AMYLASE AND PROTEASE ACTIVITIES OF SOFT RED WINTER WHEAT

(Wheats steeped to 42% moisture and germinated at 68°F. for 12 days)

VARIETY	STATION	PROTEIN	ALPHA-AMYLASE	PROTEASE
		%	*SKB units/g*	*HU/g*
Seneca	Columbus	9.9	356	106.5
	Wooster	10.4	366	112.6
Genesee	Columbus	9.2	417	109.6
	Wooster	10.4	430	112.4
Vigo	Columbus	9.0	403	105.3
	Wooster	10.9	420	110.5

Literature Cited

1. ANDERSON, J. A., MEREDITH, W. O. S., and SALLANS, H. R. Malting quality of Canadian barleys. IV. A summary of information of special interest to plant breeders. Sci. Agr. **23:** 297–314 (1943).
2. ANDERSON, J. A., SALLANS, H. R., and MEREDITH, W. O. S. Varietal differences in barleys and malts. XII. Summary of correlations between 18 major barley, malt and malting properties. Can. J. Research C**19:** 278–291 (1941).
3. BANASIK, O. J., and HARRIS, R. H. The malting quality of six North Dakota barley varieties. Brewer's Dig. **32** (11)**:** 60–65 (1957).
4. BANASIK, O. J., MYHRE, D., and HARRIS, R. H. A micromalting method for nursery samples. Brewer's Dig. **31** (1)**:** 50–55 (1956).
5. DICKSON, J. G., DICKSON, A. D., SHANDS, H. L., and BURKHART, B. A. Barley and malt studies. IV. Experimental malting of barleys grown in 1936 and summary data for three years, 1934–1936. Cereal Chem. **15:** 133–168 (1938).
6. DICKSON, J. G., and GEDDES, W. F. Effect of wheat class and germination moisture and time on the malt yield and amylase activity of malted wheat. Cereal Chem. **26:** 404–414 (1949).
7. DICKSON, J. G., SHANDS, H. L., DICKSON, A. D., and BURKHART, B. A. Barley and malt studies. V. Experimental malting of barleys grown in 1937. Cereal Chem. **16:** 468–480 (1939).
8. FLEMING, J. R., JOHNSON, J. A., and MILLER, B. S. Effect of malting procedure and wheat storage conditions on alpha-amylase and protease activities. Cereal Chem. **37:** 363–370 (1960).
9. GEDDES, W. F., HILDEBRAND, F. C., and ANDERSON, J. A. The effect of wheat type, protein content, and malting conditions on the properties of malted wheat flour. Cereal Chem. **18:** 42–60 (1941).
10. HARRIS, R. H., and BANASIK, O. J. Effects of environment, variety, and season on barley quality. Cereal Chem. **29:** 148–155 (1952).
11. KNEEN, E., and HADS, H. L. Effects of variety and environment on the amylases of germinated wheat and barley. Cereal Chem. **22:** 407–418 (1945).
12. MEREDITH, W. O. S., EVA, W. J., and ANDERSON, J. A. Effect of variety and environment on some qualities of malted wheat flour. Cereal Chem. **21:** 233–240 (1944).
13. MILLER, B. S., and JOHNSON, J. A. A simple linear relationship and definition of a unit for proteinase activity. Arch. Biochem. **32:** 200–206 (1951).

14. REDFERN, S. Methods for determination of alpha-amylase. IV. A glass end-point color standard for use in the dextrinizing method: effect of temperature and starch lot on this method. Cereal Chem. 24: 259–268 (1947).
15. SANDSTEDT, R. M., KNEEN, E, and BLISH, M. J. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. 16: 712–723 (1939).

DAMAGED STARCH. QUANTITATIVE DETERMINATION IN FLOUR[1]

R. M. SANDSTEDT AND P. J. MATTERN[2]

ABSTRACT

Damaged starch is readily and rapidly digested by the amylases, whereas the native undamaged starch is markedly more resistant to digestion. Based on these differences, a method is proposed for the quantitative determination of damaged starch in flour. The digestion of undamaged starch is appreciable and is not constant from flour to flour. Accordingly, the proposed method eliminates this digestion as a factor by determinations after 1-hour and 2-hour periods of digestion with extrapolation back to 0 time for the percent damaged starch. The slope of the curve is an indication of the susceptibility of the undamaged starch to digestion.

Starch granules are easily damaged by pressure, shear, or strain such as that applied by grinding procedures (1,8,9). The damage is particularly notable during flour milling and accordingly is of concern to the wheat milling and baking industries (4,8,16).

Many properties of doughs and of baked bread are materially affected by the quantity of damaged starch, e.g., water absorption, gassing power, handling properties of doughs, and loaf volume and tenderness of crumb in the bread (6,8,15).

Studies concerning the relation of damaged starch to these and other baking properties and studies on the milling factors responsible for starch damage have been limited by the lack of a quantitative method for its determination. The purpose of this paper is to present a method for the quantitative determination of damaged starch in flour.

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Properties of Damaged Starch As a Basis for Its Determination. Damaged starch differs from normal starch in a number of respects: water absorption (swelling in water), solubility, susceptibility to staining with iodine and certain dyes, and digestibility by amylases (5,8,9,11,13).

Damage, as evidenced by swelling and staining with dilute iodine (0.0001*N*) or Congo red does not generally extend to all parts of the granule (Fig. 1). It may be a limited local injury or it may extend throughout the granule with only small segments remaining uninjured.

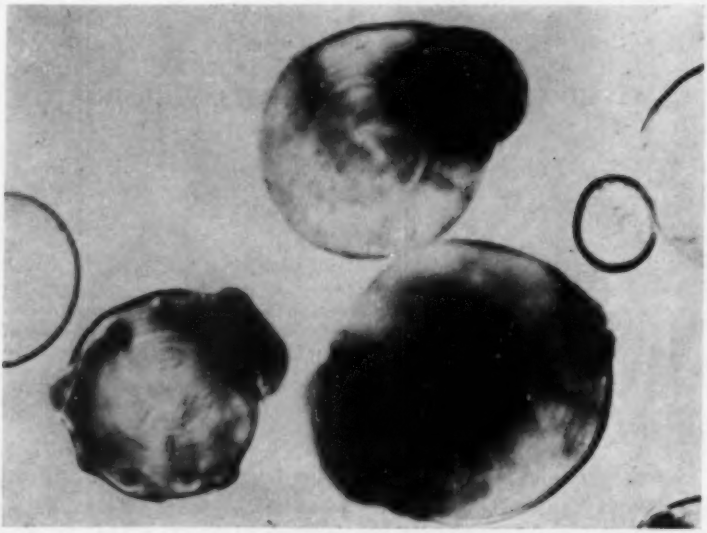

Fig. 1. Damage to starch as evidenced by swelling and staining with dilute iodine.

The variation in intensity of damage is evidenced by the amount of swelling and by the intensity of the staining with iodine in the granules of Fig. 1. Damage is not an all-or-none situation. It apparently covers the entire range from the rupture of a few intermolecular bonds, which would imperceptibly increase swelling and rate of digestion, to extensive rupture of enough bonds to make the starch readily available to amylase action. The differences in properties of damaged and undamaged starch depend both on the extent and on the degree of damage.

As a basis for the quantitative determination of damage in a flour, any of the above mentioned differences could conceivably be used. However, only the increased solubility and increased digestibility ap-

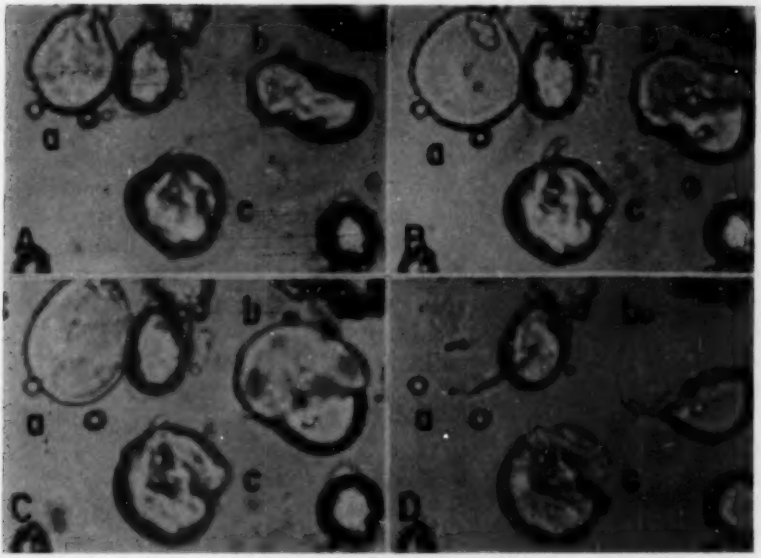

Fig. 2. Swelling and subsequent digestion of mill-damaged starch flooded with a 1:5 malt extract (16 SKB units of alpha-amylase per ml.): A, immediately after wetting (about 1/64 second), before swelling was notable; B, 0.1 second after wetting, swelling incomplete; C, one second after wetting, swelling complete; D, 30 minutes after wetting, digestion of damaged sections apparently complete. These are enlargements of selected frames from a motion picture film (13).

pear to be practical. Methods based on solubility (7) and on digestibility with beta-amylase after inactivation of the enzymes of the flour (5,6) have been proposed.

A normal starch granule is resistant to the action of digestive enzymes. Damaged starch has lost much of this resistance.

Figure 2 (13) shows the swelling and digestion of wheat flour starch after wetting with a 1:5 wheat malt extract. The observable swelling in the damaged starch was complete in less than one second. The slower subsequent digestion of the damaged starch was essentially complete in 30 minutes. At the end of this time there was no apparent action on the undamaged portions of the granules.

Rate of digestion is dependent on the accessibility of the starch molecules to the enzyme. Accordingly, slightly damaged starch may digest almost as slowly as the undamaged. The complete differentiation of damaged and undamaged starch would seem to be impractical, if not impossible. The objective in a method for the determination of damage is to determine that damage which affects the baking properties of flour; this is that degree of damage that allows appreciable

swelling and an appreciably increased digestion. The selection of conditions for such a determination is empirical and is based on the differential rate of digestion of damaged and undamaged starch.

Development of the Method

With some modifications, the ferricyanide method for the determination of maltose value (2,3,10) may be used for this determination. The use of this method for the determination of damaged starch has many advantages, since it is in general use in cereal laboratories and thus the chemicals and equipment are available and the techniques are well known. The only necessary changes in the method are the use of a relatively large quantity of a malt enzyme preparation, 1-g. samples of flour instead of 5-g., and determinations after 1- and 2-hour periods of digestion.

Quantity of Enzyme Required. To measure the damaged starch it is necessary to add enough enzyme to digest completely the readily digestible starch in a reasonably short period of time. For a 1-hour digestion this is approximately enough malt extract to supply 160 SKB units (16) of alpha-amylase. This quantity also is insurance that the relatively small quantities of enzyme naturally occurring or added to the flour during milling will be of little consequence and will cause no appreciable error.

Size of Flour Sample. The amount of maltose produced from the flour must be within the range that can be determined by the 0.1*N* ferricyanide under the conditions specified. The maltose obtained from 1-g. samples of flour by a malt extract containing 160 units of alpha-amylase were found to be within the determinable range.

Specifications for the Enzyme Preparation. Normally a malt sample carrying 160 units of alpha-amylase contains enough maltose to use up approximately half of the 0.1*N* ferricyanide. The essential elimination of this enzyme blank value may be accomplished by a thorough dialysis. Lyophilization is desirable and an alpha-amylase assay is necessary. Enough beta-amylase must be present to convert the alpha-amylase limit dextrins to maltose.[3]

Digestion-Rate Curves of a Series of Starches with Progressively Increasing Amounts of Damage. Mixtures were made of a soft wheat starch (a starch with minimum damage) with increments of a hard wheat starch which had been severely damaged by eight hours of ball-milling. The series contained 0, 10, 20, 30, 40, and 100% of the

[3] Since the lyophilization and enzyme assays are time-consuming operations, it is hoped that some commercial organization interested in enzyme preparations and in this particular work will make available a dry malt enzyme preparation standardized as to alpha-amylase value and containing only a minimum of maltose.

ball-milled starch. The digestion curves obtained from digestion of 0.75-g. samples of these starches (corresponding roughly to the starch in 1 g. of flour), with malt extract containing 160 units of alpha-amylase are shown in Fig. 3.

After 1 hour of digestion the curves are essentially parallel and approximately the same distance apart for each 10% increment of ball-milled starch; indicating that the damaged starch was digested during the first hour and that the portion of the curves, from 1 to 4 hours, represents the rate of digestion of the normal undamaged starch. The distance between curves represents the increment in quantity of damaged starch. Obviously the enzyme added was not enough for the 100% ball-milled sample, as the digestion of damaged starch was not complete in 1 hour.

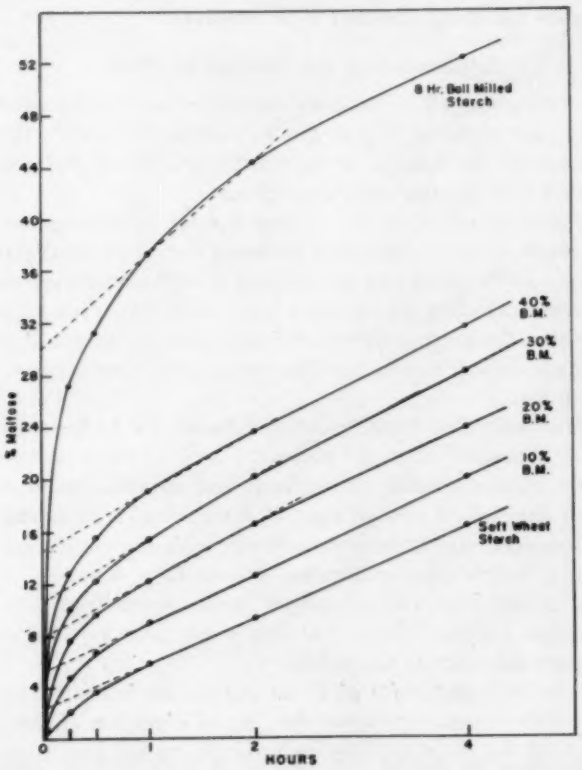

Fig. 3. Digestion-rate curves of a series of starches with progressively increasing amounts of damage.

The portion of the curves below the 1-hour point represents a combination of the rates of digestion of the damaged and the undamaged starch. The rate of digestion of the undamaged starch during this first hour is assumed to be the same as the rate from 1 to 2 hours. If the curves representing the normal starch digestion during the 1- to 2-hour period are extrapolated back to the vertical axis, the intercept at 0 time should represent the amount of maltose produced from the damaged starch.

Using malt as the enzyme source for the digestion of raw starch, the factor for conversion of maltose to starch is practically 1.0[4] (14). Accordingly, this figure for maltose from damaged starch also represents damaged starch. By this procedure the damaged starch in this series of starches was 2.4, 5.2, 8.0, 10.7, and 14.0%. The increase with each 10% increment of ball-milled starch was 2.8, 2.8, 2.7, and 3.3%. These figures make the method look feasible.

Application of the Method to Flour

The curves obtained from flours varying widely in damaged starch (3 to 18.4%) are shown in Fig. 4. The soft wheat flours show the lowest damage, whereas the damage in the hard wheat flours varies with the hardness and with milling conditions (6,8,11).

It has been found that the enzyme system of *Aspergillus oryzae* shows a much greater difference between the rates of digestion of boiled starch and that of raw starch than do the enzyme systems from other sources, including the enzymes from malt (14). This suggests the possibility that the enzyme system of *Aspergillus oryzae* would be better than the malt enzyme system for differentiation between damaged and undamaged starch.

This possibility was investigated and found not to be as expected. The curve "C-fungal" of Fig. 4 shows the type of curve and the damaged starch value obtained when the fungal enzyme system was substituted for the malt system on flour C. Flours similar to E and F with 8 to 9% damaged starch showed only 4% with the fungal enzyme.

Damaged starch has properties intermediate between those of boiled starch and those of undamaged starch. Accordingly, the action of this fungal enzyme system was much too slow on the damaged starch; it gave exceedingly low values.

Variation in Digestibility of Undamaged Starch. As before stated, the slope of the curve represents the rate of digestion of the undamaged starch of the flour. Differences in slopes of the curve also might

[4] The theoretical factor for converting maltose to starch is 0.95. Under the conditions used, the factor for conversion to damaged starch varies from 0.95 to 1.0.

indicate differences in quantity of starch that was only slightly damaged and accordingly was digested slowly. However, it would be expected that the slightly damaged starch, in general, would be in proportion to the highly damaged starch. The data of Fig. 4 indicate that this was not true for these flours. Also, if the slope of the curve were dependent on the quantity of slightly damaged starch, the curves shown in Fig. 3 (slope of curve for 1 to 4 hours) should not be parallel but should become steeper with increasing quantities of damaged starch. The wide range in slope (in digestibility) of undamaged starch from flour to flour was to be expected; it has been shown by photomicrographic methods that there are exceedingly wide variations in

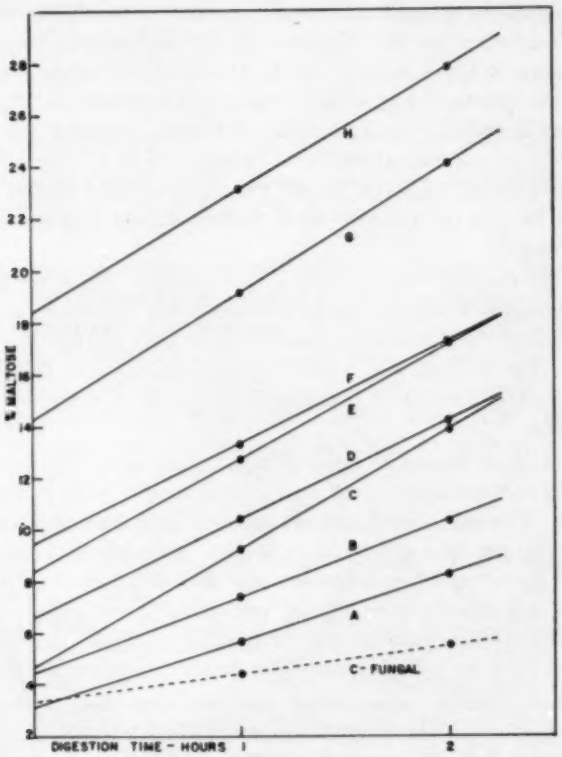

Fig. 4. Application of the method to the determination of damaged starch in flour. A, soft white wheat flour; B, soft red winter flour; C, commercial hard winter wheat flour; D, commercial spring wheat flour; E, commercial spring wheat flour; F, commercial flour; G, durum wheat flour; H, commercial air-classified flour; C-fungal, flour C digested with an enzyme system of *Aspergillus oryzae*.

rates of digestion between individual wheat starch granules (11).

For convenience the slope of the curve may be represented by one figure; the difference between the 1-hour and 2-hour determinations. It would seem that under standard conditions this figure could be used to indicate the relative digestibility of the undamaged starch. Applied to this particular group of flours: A, a soft white wheat flour, had the least digestible starch (value 2.5), and G, a durum, the most (value 5.0); whereas within classes, the two hard springs (E and D) had values of 4.4 and 3.0 and the two hard winters (C and F) had values of 4.6 and 3.4.

Use of Single Determinations vs. 1- and 2-Hour Curves. If the undamaged starch in all flours (or even in all flours of one class) were equally digestible, a single determination after 1 hour's digestion with a correction factor for the digestion of the undamaged starch, could be a measure of the damaged starch. However, the wide variation in slopes of the curves of Fig. 4 show that single values could be misleading. Flours B and C have practically the same damaged starch value but the 1-hour reading alone would give flour B a value of 7.4 and flour C a value of 9.2. The curves for the two hard spring flours D and E, as well as for the two hard winters C and F, have decidedly different slopes.

Effect of Varying the Enzyme Concentration. As is to be anticipated, variations in enzyme concentration cause variations in the rate of digestion of the undamaged starch, i. e., they change the slope of the curve. The data of Fig. 5 indicate that, in so far as the determination of damaged starch is concerned, the slope of the curve is of no consequence.

The variation from 130 units of alpha-amylase to 190 units caused no error in the damaged starch figure on a sample with a low percent of damage. The value for durum flour with 13% damage varied 0.6%. However, durum flour gives erratic results, probably because the flour is largely made up of endosperm cells and fragments of cells (12) which do not readily disintegrate under the conditions used. The 0.6% variation may indicate that the quantity of alpha-amylase should be increased for such highly damaged flours or for durum flours.

Using one enzyme preparation and the method as proposed, the standard deviation obtained from seven determinations on a soft wheat flour with 3.6% damaged starch was 0.17; from seven determinations on a durum wheat flour with 13.5% damaged starch the standard deviation was 0.20.

Continuous Shaking during Digestion. It has been suggested that

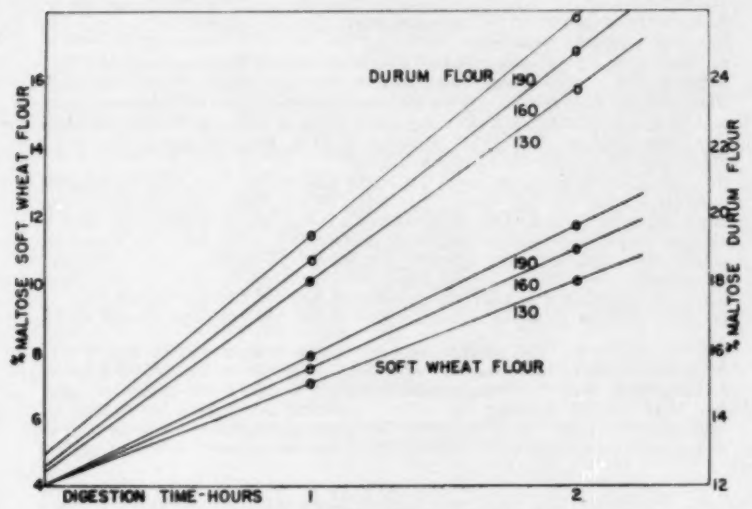

Fig. 5. The effects of varying the enzyme concentration on the amount of damaged starch found and on the slopes of the curves. Numerals 130, 160, 190 indicate the units of alpha-amylase used for the digestions.

shaking the samples during the digestion is unnecessary. Limited experience in this laboratory indicates that this may be true when using flours with relatively low amounts of damage or with soft wheat flours; on the other hand, a durum flour gave results 3 to 4% lower with no shaking than with continuous shaking. The amount of shaking also had pronounced effects on the slopes of the digestion curves. The granular structure of the hard wheat flour makes continuous shaking necessary.

Specifications for the Proposed Procedure[5]

Apparatus: (1) Thermostatically controlled water bath with a shaker.
(2) Filter paper, No. 4 Whatman or equivalent.
(3) Microburet, 10-ml. capacity.

Reagents: (1) Buffer solution. Dissolve 3 ml. glacial acetic acid and 4.1 g. anhydrous sodium acetate and make up to 1 liter with water. The pH of this solution is 4.6 to 4.8.
(2) Sulfuric acid, 3.58*N* (± 0.05). Dilute 10 ml. concentrated H_2SO_4 (sp. gr. 1.84) to 100 ml. and adjust concentration if necessary.
(3) Sodium tungstate solution, 12%. Dissolve 12.0 g. $Na_2WO_4 \cdot 2H_2O$ and dilute to 100 ml.
(4)Alkaline ferricyanide reagent, 0.1*N*. Dissolve 33 g. pure dry $K_3Fe(CN)_6$ and 44 g. anhydrous Na_2CO_3 and dilute to 1 liter. To standardize, add to 10 ml. of this solution 25 ml. acetic acid-salt solution and 1 ml. soluble starch-KI solution, and titrate with 0.1*N* thiosulfate. Exactly 10 ml. should be required to discharge the blue color completely.

[5] See reference 2.

(5) Acetic acid-salt solution. Dissolve completely 70 g. KCl and 40 g. $ZnSO_4 \cdot 7H_2O$ in 750 ml. water, add slowly 200 ml. glacial acetic acid, and dilute to 1 liter with water.
(6) Soluble starch-potassium iodide solution. Suspend 2 g. soluble starch in a small quantity of cold water and pour slowly into boiling water with constant stirring. Cool thoroughly (or the resulting mixture will be dark-colored), add 50 g. KI, dilute to 100 ml., and add 1 drop of saturated NaOH solution.
(7) Thiosulfate solution, 0.1*N*. Dissolve 24.82 g. Reagent quality $Na_2S_2O_3 \cdot 5H_2O$ and 3.8 g. borax and make to 1 liter.
(8) Malt enzyme preparation. A 1:4 malted wheat flour extract thoroughly dialyzed against distilled water (containing 20 mg. $CaCl_2$ per 100 ml.) to remove reducing sugars. The dialyzed extract may be: 1) assayed for alpha-amylase value (16) and stored at approximately 4°C. with an excess of chloroform (alpha-amylase solutions are subject to surface inactivation and accordingly they should not be vigorously shaken with the excess chloroform); 2) lyophilized and the dry powder assayed for alpha-amylase (16).
(9) Malt solution. Dissolve the equivalent of 160 SKB units per 6 ml. water.

Determination: Introduce duplicate 1-g. samples of flour and ½ teaspoonful of ignited quartz sand into 100- or 125-ml. Erlenmeyer flasks, and mix by rotating flask. Add 40 ml. buffer solution at 30°C. and 6 ml. of enzyme solution, and mix by rotating the flask. (Flask and all ingredients should be *individually* brought to 30°C. before being mixed together.) Place in a thermostatically controlled water bath at 30°C. and maintain at this temperature

TABLE I

FERRICYANIDE-MALTOSE (DAMAGED STARCH) CONVERSION

0.1*N* Ferricyanide Reduced	Maltose	0.1*N* Ferricyanide Reduced	Maltose	0.1*N* Ferricyanide Reduced	Maltose
ml	*% of flour*	*ml*	*% of flour*	*ml*	*% of flour*
0.10	0.25	3.10	7.80	6.10	17.1
0.20	0.50	3.20	8.05	6.20	17.4
0.30	0.75	3.30	8.30	6.30	17.7
0.40	1.00	3.40	8.55	6.40	18.1
0.50	1.25	3.50	8.80	6.50	18.4
0.60	1.50	3.60	9.10	6.60	18.7
0.70	1.75	3.70	9.40	6.70	19.0
0.80	2.00	3.80	9.70	6.80	19.3
0.90	2.25	3.90	10.1	6.90	19.6
1.00	2.50	4.00	10.4	7.00	19.9
1.10	2.75	4.10	10.7	7.10	20.3
1.20	3.00	4.20	11.0	7.20	20.6
1.30	3.25	4.30	11.3	7.30	21.0
1.40	3.50	4.40	11.6	7.40	21.3
1.50	3.75	4.50	11.9	7.50	21.6
1.60	4.00	4.60	12.2	7.60	22.0
1.70	4.25	4.70	12.6	7.70	22.3
1.80	4.50	4.80	12.9	7.80	22.6
1.90	4.75	4.90	13.2	7.90	23.0
2.00	5.00	5.00	13.5	8.00	23.3
2.10	5.25	5.10	13.8	8.10	23.6
2.20	5.50	5.20	14.1	8.20	24.0
2.30	5.75	5.30	14.4	8.30	24.3
2.40	6.00	5.40	14.8	8.40	24.6
2.50	6.25	5.50	15.2	8.50	25.0
2.60	6.50	5.60	15.5	8.60	25.3
2.70	6.75	5.70	15.8	8.70	25.7
2.80	7.00	5.80	16.2	8.80	26.0
2.90	7.25	5.90	16.5		
3.00	7.55	6.00	16.8		

for exactly 1 hour for the first sample and 2 hours for the second, shaking the flasks continuously. The shaking should only be enough to keep the flour in suspension. At the end of the 1-hour and 2-hour periods add 2 ml. 3.58*N* H_2SO_4 to the respective flasks and mix thoroughly. Add immediately 2 ml. sodium tungstate solution and again mix thoroughly. Let stand 2 minutes and filter (No. 4 Whatman or equivalent), discarding the first 8 to 10 drops of filtrate.

Pipet a 5-ml. aliquot of the filtrate into a test tube of approximately 50-ml. capacity (length 20 cm., diameter 2 cm.). Add with a pipet exactly 10 ml. alkaline ferricyanide reagent, mix, and immerse the test tube in a vigorously boiling water bath. The surface of the liquid in the test tube should be 3 to 4 cm. below the surface of the boiling water. (The delay between the filtering of the extract and the treatment in the boiling water bath should not exceed 15 to 20 minutes. Further delay may cause error due to fructan hydrolysis in the acid solution.) Allow the test tube to remain in the boiling water bath exactly 20 minutes. Cool the test tube and contents under running water and pour at once into a 100- or 125-ml. Erlenmeyer flask. Rinse out the test tube with 25 ml. acetic acid-salt solution reagent, adding rinsings to the solution in the Erlenmeyer flask. Mix, add 1 ml. of soluble starch-KI solution, mix thoroughly and titrate with 0.1*N* thiosulfate to the complete disappearance of the blue color. (A 10-ml. microburet is recommended for this titration.) Calculate ml. ferricyanide reduced by subtracting ml. thiosulfate required for the determination from the thiosulfate used for the enzyme-reagent blank. Convert to maltose by means of the Ferricyanide-Maltose (Damaged Starch) Conversion Table (Table I). Plot percent maltose against digestion time, drawing a straight line from the vertical axis at 0 time through the 1-hour and 2-hour points. The intercept with the vertical axis at 0 time is the percent maltose produced from damaged starch or percent damaged starch.

Literature Cited

1. Alsberg, C. L., and Griffing, E. P. Effect of fine grinding upon flour. Cereal Chem. **2:** 325–344 (1925).
2. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.), pp. 122–124. The Association: St. Paul, Minn. (1957).
3. Blish, M. J., and Sandstedt, R. M. An improved method for the estimation of flour diastatic value. Cereal Chem. **10:** 189–202 (1933).
4. Blish, M. J., Sandstedt, R. M., and Kneen, E. The cereal amylases with reference to flour and malt behavior. Cereal Chem. **15:** 629–657 (1938).
5. Dadswell, Inez W., and Gardner, Joan F. The relation of alpha-amylase and susceptible starch to diastatic activity. Cereal Chem. **24:** 79–99 (1947).
6. Greer, E. N., and Stewart, B. A. The water absorption of wheat flour: Relative effects of protein and starch. J. Sci. Food Agr. **10:** 248–252 (1959).
7. Hampel, G. Die exakte Bestimmung der Stärkebeschädigung und des Totmahlens durch die Amylosezahl. Getreide u. Mehl **2:** 16–19 (1952).
8. Jones, C. R. The production of mechanically damaged starch in milling as a governing factor in the diastatic activity of flour. Cereal Chem. **17:** 133–169 (1940).
9. Pulkki, L. H. Particle size in relation to flour characteristics and starch cells of wheat. Cereal Chem. **15:** 749–765 (1938).
10. Sandstedt, R. M. The adaptation of the ferricyanide maltose method to high diastatic flours. Cereal Chem. **14:** 603–604 (1937).
11. Sandstedt, R. M. Photomicrographic studies of wheat starch. III. Enzyme digestion and granule structure. Cereal Chem. **31:** Suppl. (1954).
12. Sandstedt, R. M., and Fleming, J. Milling properties of wheat. Relation to endosperm structure and hardness. Cinemicrograph, University of Nebraska, Lincoln (1952). Presented at the 37th annual meeting, AACC (1952).
13. Sandstedt, R. M., and Fleming, J. The swelling and enzyme digestion of mechanically injured wheat starch granules. Cinemicrograph. University of Nebraska, Lincoln (1954). Presented at 125th annual meeting, American Chemical Society (1954).

14. SANDSTEDT, R. M., and GATES, R. L. Raw starch digestion: A comparison of the raw starch digesting capabilities of the amylase systems from four alpha-amylase sources. Food Research **19**: 190–199 (1954).
15. SANDSTEDT, R. M., JOLITZ, C. E., and BLISH, M. J. Starch in relation to some baking properties of flour. Cereal Chem. **16**: 780–792 (1939).
16. SANDSTEDT, R. M., KNEEN, E., and BLISH, M. J. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723 (1939).

WEIGHT AND VOLUME CHANGES IN WHEAT DURING SORPTION AND DESORPTION OF MOISTURE[1]

W. BUSHUK AND I. HLYNKA

ABSTRACT

The toluene-displacement pycnometric method was applied to measure the change in volume of whole wheat with gain or loss of moisture after equilibration at various relative pressures of water vapor. The corresponding weight changes were determined also. A plot of the volume changes against the equilibrium relative pressure gave a *volume* isotherm with hysteresis between the sorption and the desorption branches similar to that in the *weight* isotherm.

A definite hysteresis also exists between the sorption and the desorption branches of the plots of change in volume against change in weight. The sorption branch seems to be curvilinear. At low moistures, the weight changes faster than the volume; at intermediate moisture the change in volume varies directly with the change in weight; and, at moistures above about 15% (dry basis) the volume changes faster than the weight. There seems to be a net decrease in total volume for low sorption and a net increase for high sorptions. For the desorption branch the change in volume varies directly with the change in weight. The dry grain shows a net increase in volume after a complete sorption-desorption cycle in the relative pressure range 0 to 0.94.

Wheat density changes approximately linearly with moisture for both branches of the isotherm. The densities during desorption are lower than the values during sorption at equivalent relative pressures. Wheat which was put through a sorption-desorption cycle in the moisture range 0 to 26.4% showed a net decrease in density of 0.014 g. per ml.

The standard method in sorption studies is to measure the increase or decrease in weight of the sorbent equilibrated to various pressures of the sorbate. Such data give a *weight* isotherm, the shape of which gives some indication of the mechanism of the sorption process. A less common approach is to measure the volume changes of the sorbent during uptake or loss of sorbate. These data can be used to construct a *volume* isotherm. This isotherm may yield information

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on the sorption process additional to that obtainable from the weight isotherm.

This paper reports the results of a study in which the weight and volume isotherms were obtained under identical conditions for the sorption and desorption of water vapor by wheat. The variations in density of the wheat used, with moisture content, during sorption and desorption, which can be derived from the weight-volume data are reported and discussed also.

Materials and Methods

The wheat used in this study was a high-grade sample of hard spring wheat from which all small and broken kernels were removed by hand picking. Its protein content (N × 5.7) was 15.7% on a 14% moisture basis.

The following method was used: Two approximately 4-g. samples of wheat, one for volume determination and the other for moisture content determination, were equilibrated above constant humidity solutions of sulfuric acid in vacuum desiccator chambers from which the air was removed by evacuation. Under such conditions equilibration periods of 3 to 4 days were sufficient to establish moisture equilibrium (2). The vapor pressures of the sulfuric acid solutions were obtained from reference tables, using the densities of the solutions which were determined pycnometrically.

To ensure that the changes in moisture content and volume were unidirectional during sorption and desorption, the samples for the sorption experiments were initially vacuum-dried at room temperature to 1.5% moisture; the samples for the desorption experiments were equilibrated to 26.4% moisture.

The entire experiment was carried out in a temperature-controlled room set at 22.2°C. All weighings were made in air.

Moisture contents (weight changes) of the wheat samples were determined from the loss in weight after drying in a vacuum oven for 24 hours at 22.2°C., 48 hours at 40°C., and finally at 100°C. until the weight changed by less than 0.001 g. after an evacuation period of 1 hour at this temperature. The final drying step usually required about 4 hours. This method was adopted instead of the standard AACC method (1) for wheat to eliminate possible moisture interchange during grinding, particularly for the low- and high-moisture samples. However, moisture data obtained by the two methods were comparable for check samples containing from 8 to 14% moisture.

Volumes were determined from the amount of toluene displaced from calibrated, Weld-type pycnometers, using the density of toluene

at the temperature of the experiment. The filled pycnometers were equilibrated in a water bath at 22.20° ± 0.02°C.

All weight and volume changes given in this paper are on a dry matter basis. The wheat was assumed dry when dried by the procedure used to determine moisture content. Densities were calculated as outlined by Sharp (12).

Results and Discussion

Representative data from approximately 20 determinations for each of the sorption and the desorption cycles are summarized in Table I. These points were selected to give a good distribution of points over the relative pressure range investigated. The first and second columns give the relative water-vapor pressures and the corresponding equilibrium weight changes. The volume changes in column 3 are the net changes per g. of dry matter obtained by subtracting the specific volume at 0% moisture from the volumes, per g. of dry matter, at the other moistures.[2] The densities in column 4 are for 22.2°C. and were calculated using the density of toluene at the same temperature.

Figure 1 gives the standard sorption-desorption isotherm (weight) as well as the volume isotherm which relates the change in volume of

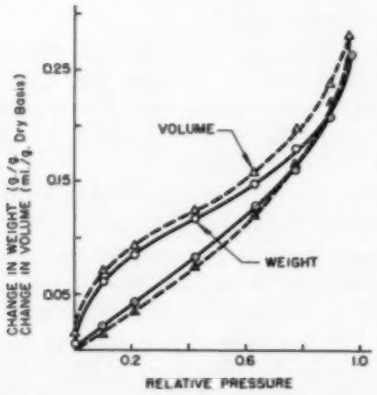

Fig. 1. Sorption isotherms for water vapor and wheat. Lower curves, sorption; upper curves, desorption.

[2] Sample calculation of the volume change at 0.77 relative pressure for the desorption cycle:
Weight of wheat (wet), 4.5413 g.
Volume of wheat (wet), 3.456 ml.
Moisture content (dry basis), 18.0%
Dry matter in 4.5413 g. of wet wheat = 4.5413/1.18 = 3.8486
Volume per g. of dry matter = 3.456/3.8486 × 1 = 0.898 ml.
Volume per g. of dry matter at 0% moisture = 0.701 ml.
Change in volume per g. = 0.898 − 0.701 = 0.197 ml. per g. of dry matter.

TABLE I

WEIGHT, VOLUME, AND DENSITY CHANGES IN WHEAT DURING SORPTION AND DESORPTION OF WATER VAPOR

Relative Vapor Pressure	Weight Change	Volume Change	Density
	g/g, dry basis	*ml/g, dry basis*	*g/ml*
Sorption: Initial moisture = 1.5%, dry basis			
0[a]	0	0	1.426
0.10	0.022	0.016	1.423
0.21	0.043	0.035	1.417
0.42	0.083	0.075	1.396
0.63	0.128	0.120	1.374
0.77	0.162	0.165	1.342
0.89	0.207	0.220	1.311
0.94	0.264	0.285	1.282
Desorption: Initial moisture = 26.4%, dry basis			
0.94	0.264	0.285	1.282
0.89	0.207	0.235	1.290
0.77	0.180	0.197	1.314
0.63	0.147	0.157	1.337
0.42	0.118	0.127	1.350
0.21	0.086	0.094	1.366
0.10	0.063	0.070	1.379
0 (Drierite)	0.005	0.014	1.406
0 (Oven-dried)	0.0	0.008	1.412

[a] Dried in oven after vacuum-drying to 1.5% moisture.

1 g. of dry wheat during gain or loss of moisture as a function of the equilibrium relative pressure.

The two branches of the weight isotherm are of sigmoid shape and essentially the same as those published by Babbitt (2) and Becker and Sallans (5). This isotherm is included here for the purpose of comparison with the volume isotherm. The sorption branch of the volume isotherm appears to be convex to the pressure axis over the entire range investigated. The desorption branch is of sigmoid shape similar to the corresponding branch of the weight isotherm.

Figure 1 shows two interesting features that might be emphasized. First is the much greater hysteresis effect in the volume isotherm than in the weight isotherm and the definite indication that after desorption from 26.4% moisture there is a residual increase in volume of the wheat, indicating that a portion of the volume hysteresis is irreversible. This is probably due to micro cracks, the formation of which has been demonstrated in moistened and dried wheat (7), in naturally and artificially weathered wheat (10), and in conditioned wheat (8). Practical implications of this observation will be discussed later.

The second interesting feature is the relation of the volume chang-

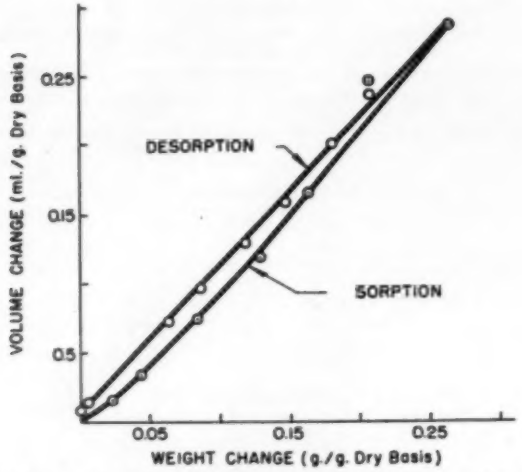

Fig. 2. The change in volume as a function of the change in weight at the same relative pressures for water vapor and wheat.

es to the weight changes. Figure 2, which gives the relation between volume change and weight change at the same relative pressure for the two branches of the isotherm, is included to facilitate discussion of this feature. Since there is no theoretical reason at present to believe that there shoud be a crossover of the sorption and desorption curves for the volume change versus weight change at 0.89 relative vapor pressure, it was assumed that deviation of the one point for sorption was due to a combination of experimental error. (See discussion of errors below.)

For the sorption portion of the cycle, the plot of volume increase against weight increase is curvilinear, whereas the corresponding plot for the desorption branch appears to be linear. There is a definite hysteresis between the sorption and the desorption branches and also a residual volume change in the wheat after the sorption-desorption cycle is completed. A similar volume hysteresis was observed by Barkas (3) in the volume changes of wood fibers during sorption-desorption of moisture.

In a study of this type where significance is attached to relatively small differences in the quantities measured, it is necessary to ensure that the differences are not invalidated by the magnitude of the experimental errors. The largest source of error in working with wheat is the sampling error. An attempt was made to reduce this as much as possible in this study by using a large sample of sound kernels. An-

other source of error in the volume determination is the entrapment of air in the creases and larger cracks that would otherwise be accessible to toluene. Most of such air was generally removed by tapping the filled pycnometer until no more bubbles could be removed. The subsequent replicability of the method was checked by determining the volume and weight changes in six replicate samples of wheat conditioned at the same time in a single desiccator. The specific volume changes calculated from the measured volumes of wheat samples which varied in weight from 4.433 to 4.654 g. were (0.121 ± 0.002) ml. per g. The corresponding densities were (1.372±0.003) g. per ml. Six replicate moisture determinations by the method outlined above gave values that were within ± 0.2% from the average value, that is, the weight change was reproducible to ±0.002 mg. per g. at a moisture level of 12%. No comprehensive statistical study was made of the errors at low and high moisture contents. Experience indicates that the errors in these regions would be slightly higher. At high relative pressures, development of mold was occasionally a source of difficulty. Samples that showed evidence of moldiness were discarded. On the basis of this semiquantitative appraisal of the experimental errors involved, it was concluded that the relative trends of the results shown in Table I are definitely significant.

The curvilinear plot for volume change against weight change for the sorption branch (see Fig. 2) can be explained as follows. Initially, the weight change is much greater than the corresponding volume change. Consequently, there is a net decrease in the total volume of the system. This physical effect is probably a direct manifestation of the high energy attraction forces between the sorption sites and the water molecules. The high heat of sorption for the low moisture levels (6) supports this hypothesis. This region of negative volume change (based on total volume) is followed by a region where the volume change seems to be directly proportional to the weight change. At moisture above about 15%, the volume seems to change much faster than the weight change so that there is actually a crossover of the sorption branches of the isotherms (see Fig. 1). It is postulated that this relation arises from the rapid swelling of the wheat that occurs at high moistures. This seems to be accompanied by a permanent, physical change in the kernel which probably is responsible for some of the hysteresis between the sorption and the desorption branches of the isotherm (2), the change of vitreous to starchy of the wheat-endosperm appearance (13), and the appearance of fissures (8,10). The consequence of this physical change is a net change in density which has been measured previously (10) to a limited extent and determined

over a relatively wide moisture range for both branches of the isotherm in the present study.

The theoretical significance of the results presented in this paper concerns their relation to the mechanics of the sorption-desorption process. It appears that any explanation of the shape of the isotherm and the hysteresis effect must include the changes in the physical structure of the wheat kernel during moisture interchange. Barkas (4) has suggested that the structural changes (plastic deformation) of the gel-type sorbent is responsible for the hysteresis in the system comprising water vapor and wood fiber. The sorptive properties of wheat are quite similar to those of wood fiber. On the basis of the hypothesis of Barkas (4) it can be predicted that the degree of sorption hysteresis should decrease as the sorbent particle size decreases. The hysteresis loops for wheat (2), for farina[3], for flour (6), and for ball-milled flour[4] decrease in the order named and, accordingly, are in line with this prediction.

In addition, Barkas (3) actually developed a theory showing that systems which exhibit sorption hysteresis owing to plastic deformation will also show a marked volume hysteresis such as is shown for wheat and water vapor in Fig 2. It is probable that the plastic deformation in the sorbent not only relieves the swelling stresses but also uncovers sorption sites which are not available at the lower relative pressures where the stress is insufficient to overcome the structural strength. The additional sorption sites may be responsible for at least a part of the rapid increase in the amount of sorption at relative pressures above about 0.6. This rapid increase in sorption is generally attributed to capillary condensation.

The practical significance of the present study concerns the residual change in density of wheat when it is put through a complete sorption-desorption cycle. The variation of wheat density with moisture content is well established (9,12); however, there are only limited published data (11) of densities for both sorption and desorption over a wide range of moistures. Figure 3 gives the variation of kernel density with moisture for the two types of moisture interchange obtained in the present study. The divergence in density for the two isotherm branches is quite apparent; however, it should be emphasized that this divergence can only be readily demonstrated if the wheat that is used for the sorption experiments is equilibrated initially to a very low moisture content; and conversely, the wheat for the desorption study must initially be at the highest moisture level used.

[3,4] Unpublished results.

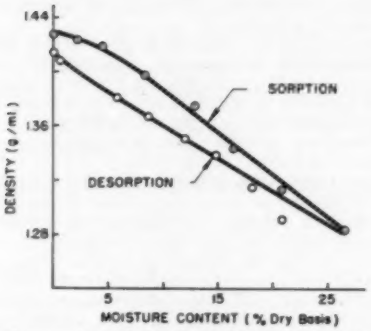

Fig. 3. Variation of gross kernel density with moisture content during sorption and desorption.

The net decrease in density of wheat that has been wetted and dried will give a decrease in the test weight and may actually change the grade of the wheat. For example, the change in bushel weight due to change in the density alone that will result when wheat of 7% moisture is wetted to 17% moisture and dried back to 7%, interpolated from Fig. 3, would be about 0.5 lb. This value will increase as the moisture range increases.

In conclusion, it is of interest to compare the density values obtained in this study with published values, particularly those of Sharp (12) and Jones and Campbell (9). The method used in this study is identical to that of Sharp (12), but the densities obtained in the present study for the sorption branch are slightly lower. The difference is probably due to the difference in the starch-protein ratio of the wheats used. The values of Jones and Campbell (9), obtained by flotation of small endosperm particles, are considerably higher than the values given in this paper. This difference can be due to two possible reasons in addition to the difference in protein content. First is that the density of the endosperm is higher than that of the other kernel components, and second is the difference in the methods used. The toluene-displacement method with whole kernels gives a gross kernel density, whereas the technique of Jones and Campbell (9), which uses small endosperm particles, is probably closer to the true density. If the wheat kernel has any internal cracks or fissures which are not accessible to toluene, then the gross density would tend to be lower than the true density.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).

2. Babbitt, J. D. Observations on the adsorption of water vapour by wheat. Can. J. Research **27F:** 55–72 (1949).
3. Barkas, W. W. Hysteresis in the swelling of gels. Nature **162:** 32–33 (1948).
4. Barkas, W. W. Wood-water relationships. VII. Swelling pressure and sorption hysteresis in gels. Trans. Faraday Soc. **38:** 194–209 (1942).
5. Becker, H. A., and Sallans, H. R. A study of the desorption isotherms of wheat at 25°C. and 50°C. Cereal Chem. **33:** 79–91 (1956).
6. Bushuk, W., and Winkler, C. A. Sorption of water vapor on wheat flour, starch, and gluten. Cereal Chem. **34:** 73–86 (1957).
7. Campbell, J. D., and Jones, C. R. On the physical basis of the response of endosperm density to change in moisture content. II. Reversibility of endosperm density on drying in relation to behavior of whole-wheat grains. Cereal Chem. **32:** 333–343 (1955).
8. Grosh, G. M., and Milner, M. Water penetration and internal cracking in tempered wheat grains. Cereal Chem. **36:** 260–273 (1959).
9. Jones, C. R., and Campbell, J. D. Micro-determination of endosperm density as a means of mapping moisture distribution in wheat grains. Cereal Chem. **30:** 177–189 (1953).
10. Milner, M., and Shellenberger, J. A. Physical properties of weathered wheat in relation to internal fissuring detected radiographically. Cereal Chem. **30:** 202–212 (1953).
11. Scott, J. H. Flour milling processes. Chapman & Hall: London (1951).
12. Sharp, P. F. Wheat and flour studies. IX. Density of wheat as influenced by freezing, stage of development, and moisture content. Cereal Chem. **4:** 14–46 (1927).
13. Swanson, C. O. Effects of moisture on the physical and other properties of wheat. Cereal Chem. **18:** 705–729 (1941).

IMPROVED TEMPERING AND MODIFIED MILLING TECHNIQUES FOR SMALL SAMPLES OF WHEAT[1]

L. D. Sibbitt,[2] D. H. Classon,[2] and R. H. Harris[2]

ABSTRACT

A rotary shaker is described for tempering wheat samples for a modified short milling procedure which permits milling 80 or more 100–150 g. samples per day by one operator. The method requires a shaking time of 15 minutes, and twelve samples can be tempered each 7.5 minutes. A micro mill was more suitable than the standard Allis mill for this work, since the micro mill flour yield was approximately 25% higher, resulting in a saving of both wheat and time required.

In genetic and inheritance studies with wheats, as well as in wheat breeding programs, mounting pressure has been brought to bear on the cereal technologist to increase the number of samples milled per day. Coupled with this pressure is the need for tests on a smaller sample, so that some concept of quality is available at an earlier stage in the breeding program.[3] This led to a study of the possibility of

[1] Published with the approval of the Director, North Dakota Agricultural Experiment Station.
[2] North Dakota Agricultural Experiment Station, Fargo, North Dakota.
[3] Various methods were examined, one a quick method developed by the North Dakota State Mill & Elevator using an Allis Experimental mill. This required a relatively large amount of wheat and would be unsuitable for nursery samples. (Private communication.)

shortening the procedure used in this laboratory for milling nursery samples with a micro mill and resulted in what seems to be a satisfactory modification. Although the method developed requires a substantial amount (over 100 g.) of wheat, it permits many more samples to be milled in one day than the micro method described by Sibbitt, Scott, and Harris (4).

The increased number of samples possible to mill in a day by the modified method focused attention on the problem of tempering. This department customarily added the required quantity of distilled water and stirred the wheat manually. This method consumed the entire attention of the technologist, and probably also lacked uniformity. Consequently the procedure was modified.

This paper describes the method and apparatus involved in tempering and milling and includes some representative data obtained from milling hard red spring wheats by the modified procedure.

Materials and Methods

Tempering. A modification of the apparatus described by Seeborg *et al.* (5) was not satisfactory because of the limited number of samples handled. A rotary mixer capable of treating at least 10 to 12 samples at one time was a necessity. Tempering by placing the sample in an open container exposed to refrigerated and relatively humid air, as was done by Seeborg when tempering 5-g. samples, would entail a much longer time for 100-g. quantities than was desirable, and would mean a number of stirrings to ensure uniform moisture distributions within each sample.

Preliminary tests using the rotary shaker described by Harris and Johnson (3) indicated that a faster rate of revolution was required than 14 r.p.m., and construction of the shaker itself was not well adapted for the number of samples milled by the modified method. Therefore a larger mixing unit was devised, capable of holding twelve jars of wheat. After a number of experiments a rotation rate of 24 r.p.m. and a mixing time of 15 minutes was selected as satisfactory.

Figure 1 is a line drawing of the apparatus finally adopted for tempering the samples for fast micromilling. Essentially this consists of two frames enclosed on three sides but open on the remaining side to facilitate inspection and removal of the samples, which were enclosed in glass 1-pt. jars. The top of each frame was hinged along the back and fastened with metal clips at the front. A support was fastened in the center of each for extra strength. The bottom and top were recessed to hold the jars firmly. Twenty-four jars can be accom-

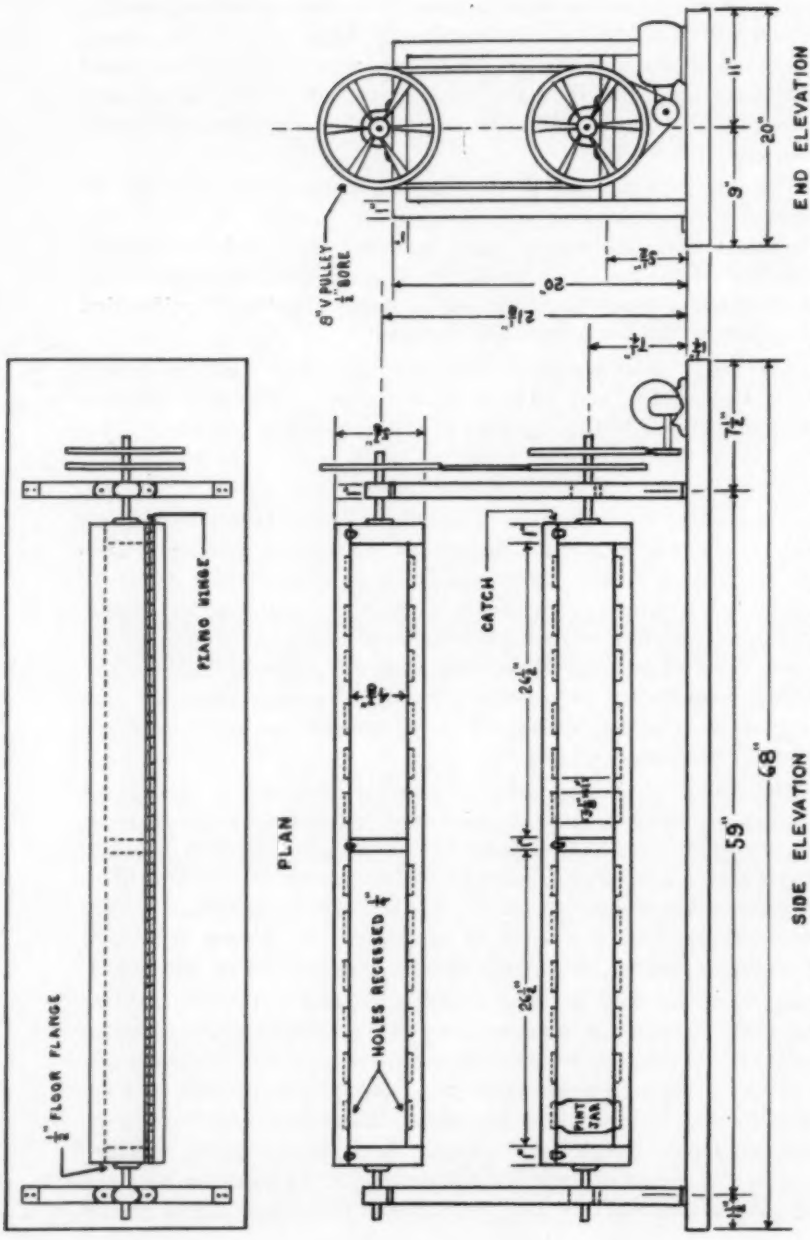

Fig. 1. Tempering apparatus for wheat samples for the modified micromilling procedure.

modated at one time. A 1/12 h.p. motor with attached reduction gear served to rotate the frames at 24 r.p.m. The upper unit was driven from the lower by a pulley and belt. By disconnecting the belt, the lower one could be operated independently. For tempering, a dispenser with finger-tip control connected to a suitable reservoir proved advantageous.

Tempering was as follows: To the weighed wheat samples sufficient distilled water was added to attain a final moisture content of 16.3%. The sample was then shaken thoroughly by hand. Twelve samples were thus treated, then placed in the mixer. Mixing was continued for 7½ minutes, after which the machine was stopped, and each sample jar turned to remove kernels adhering to the sides of the jars. Twelve additional samples, which had been tempered while the first twelve were mixing, were now placed in the upper unit. The lid was then fastened and mixing resumed for another 7½ minutes, yielding a total of 15 minutes of mixing for the first lot, which were now removed from the mixer, shaken for the second time by hand, and set aside until the following morning for milling. The second lot of twelve sample jars was also turned in the mixer as for the first lot of samples, and a third set of twelve tempered samples placed in the machine and rotation resumed. Thus twelve samples can be tempered every 7½ minutes by this procedure after the first 15-minute period.

Milling. A small-scale experimental mill as described by Geddes and Frisell (1) was used. This mill consisted of a bolter, one set of break rolls, and one set of reduction rolls. A modification of the micromilling method described by Geddes and Aitken (2) and Sibbitt, Scott, and Harris (4) was made; this consisted of reducing the number of breaks and reductions to yield a shorter-extraction flour. The milling time per sample was decreased from approximately 20 to 5 minutes, increasing the number milled to approximately 80 per day. The flow

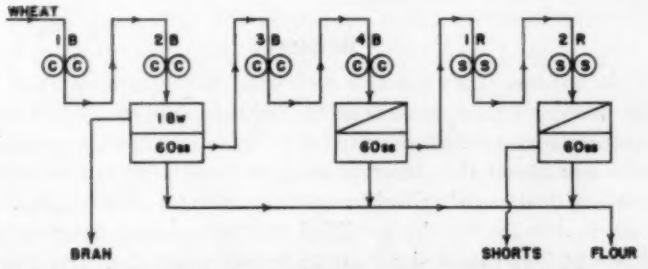

Fig. 2. Flow sheet used in the modified micromilling procedure for hard red spring wheats. W = wire; SS = stainless steel.

sheet used is shown in Fig. 2. It consists of four breaks and two reductions, with bolting after only the second and fourth break and the second reduction. First break rolls were set 0.020 in. apart. The material was then repassed through the break rolls at the same setting and then bolted, using 18W and 60SS sieves. Bran remaining on the 18W sieve was discarded. Material on the 60SS sieve was ground on the third break, using a roll spacing of 0.003 in., then passed through the fourth break at the same setting without bolting. The stock was then sifted through the 60SS sieve and the material remaining on the sieve passed through the first reduction with the rolls barely touching. The material was then passed through the second reduction at the same roll spacing. Material remaining on the 60SS sieve which consisted of shorts and low-grade flour was discarded. The throughs consisted of the 50% extraction flour.

Abnormal milling characteristics were easily detected by this new method. Preliminary studies made on a series of wheats by both the standard micromilling procedure and the modified rapid method showed a good relationship between the quality factors examined.

The baking method employed was malt-phosphate-bromate with 5% sucrose and a 3-hour fermentation period (4). The 25-g. flour doughs were mixed in a National micro mixer using variable mixing times.

The method used for evaluation of the mixograms is one developed in this laboratory and consists of a series of eight standard curves which range from a "very strong" to an "extremely weak" classification. A numerical score is assigned to each classification to facilitate obtaining an average figure when necessary. Included in these standards are the mixing time, mixing tolerance, and band width. An unknown curve is compared against the standards and the proper classification assigned. This procedure is very rapid, eliminates the necessity of measuring each individual curve, and gives a single-figure score, which is most desirable.

Results

Table I shows representative data obtained from a series of flours milled by this method. Several of the hybrids were not baked because of unsatisfactory mixing charateristics. In a previous (unpublished) study it was found that flour protein and ash content corresponded very well with those obtained from flour milled by the original micro method. Naturally, by the modified milling method flour yield was much lower, but varied significantly between samples. The flour was darker and more specky. Loaf volume and crumb color tended to be

TABLE I

MISCELLANEOUS DATA ON SAMPLES MILLED BY THE MODIFIED METHOD

1958 Lab. No.	Wheat Protein [a]	Flour Protein [a]	Flour Ash [a]	Flour Yield	Mixogram Classification [b]	Loaf Volume	Crumb Color [c]
	%	%	%	%		cc	
197	14.8	13.9	0.45	53.7	3	200	7.0
203	14.4	13.4	0.38	52.5	6	135	6.0
215	15.9	15.0	0.46	49.7	4	190	6.0
218	16.5	15.6	0.45	47.1	3		
221	15.2	14.6	0.45	46.7	3		
230	16.0	14.8	0.46	49.9	2		
236	16.3	15.4	0.49	46.5	4	185	5.5
244	16.7	16.3	0.52	52.0	4	175	5.5
253	16.0	15.1	0.43	46.5	7	150	6.0
259	15.6	14.1	0.44	51.3	5	145	5.5
266	16.4	14.7	0.43	51.9	4	175	6.0
271	17.0	15.7	0.45	51.1	3	220	7.0
280	15.3	14.5	0.49	48.3	4	180	6.0
296	16.6	15.5	0.52	47.1	2		
302	13.8	12.7	0.41	51.2	7	195	6.0
307	13.4	12.2	0.45	52.5	6	180	6.0
309	13.4	11.2	0.41	47.5	6	175	7.5
311	13.5	11.8	0.49	53.4	6	185	5.5
314	13.7	12.6	0.43	54.7	5	200	5.5
319	13.0	12.3	0.56	49.5	6	180	5.5
323	14.0	12.8	0.48	54.8	6	195	8.0
325	13.7	12.4	0.46	49.5	4	185	6.5
330	13.6	12.2	0.43	53.1	6	180	7.5
333	14.3	12.7	0.46	53.1	6	190	6.5
338	13.7	12.4	0.46	53.3	4	185	5.5
340	13.9	12.5	0.46	53.1	4	185	7.0
342	13.5	12.4	0.46	48.7	5	175	6.0
344	14.1	12.5	0.49	49.5	3	170	5.5
Mean	14.8	13.6	0.46	50.7	4.5	181	6.2

[a] Expressed on 14.0% moisture basis.
[b] Mixogram classifications: 1, 2, 3 are unsatisfactory curve types; 4, 5 are questionable curve types; 6, 7, 8 are satisfactory curve types.
[c] Crumb color: perfect score 10.0.

below the values of flour milled by other procedures. Mixogram patterns were determined at a constant absorption level, and resembled those obtained from the flour produced by the standard micromilling method using the baking absorption.

For early testing and screening of wheat samples the modified method should be more suitable than the original procedure. More samples can be milled in one day, and a slightly smaller quantity of wheat is needed. It is particularly advantageous for obtaining an early concept of mixing requirements, and assists in determinations of probable baking quality.

The method was also used with the Allis to see whether this mill could be substituted for the micro mill, since most cereal laboratories

TABLE II

RESULTS OBTAINED FROM ALLIS MILL WITH MODIFIED METHOD

SAMPLE WEIGHT	MILLING TIME		YIELD[a]	
g	*minutes*	*seconds*	*g*	%
2000	14	20	655.0	32.8
1000	8	15	320.0	32.0
500	7	5	180.0	36.0
200	5	55	57.8	23.9
150	4	30	43.0	28.6
100	4	25	27.5	27.5

[a] Bran high in endosperm content.

do not have the latter. The results obtained with samples of various sizes are shown in Table II. While the time required to mill 200 g. or less of wheat corresponded closely to that of the micro mill, the flour yield was much lower (by about 25%). At least 200 g. of wheat would be needed to yield sufficient flour for mixogram and baking tests, and this increases the milling time per sample approximately 1 minute. The more important consideration, however, is the larger quantity of wheat necessary to produce sufficient flour for adequate testing.

It was not possible to clean up the bran, and this contributed to the low flour yield. The flour compared satisfactorily with that produced in the micro mill. On the third and fourth break a reduced feed rate was required to prevent flaking. Somewhat more time was found necessary to clean the mill between samples and remove adherent flour from the mill interior than with the micro mill.

Literature Cited

1. GEDDES, W. F., and FRISELL, B. An experimental flour mill for 100-gram wheat samples. Cereal Chem. **12:** 691–695 (1935).
2. GEDDES, W. F., and AITKEN, T. R. An experimental milling and baking technique requiring 100 grams of wheat. Cereal Chem. **12:** 696–707 (1935).
3. HARRIS, R. H., and JOHNSON, JOHN, JR. Some physical and chemical changes occurring in hard red spring wheat gluten during dough fermentation. Cereal Chem. **17:** 739–759 (1940).
4. SIBBITT, L. D., SCOTT, G. M., and HARRIS, R. H. A further comparison of flours obtained with the micro and Allis-Chalmers mills. Cereal Chem. **20:** 679–685 (1943).
5. SEEBORG, E. F., SHOUP, N. H., and BARMORE, M. A. Modification of the Buhler mill for micro milling. Cereal Chem. **28:** 299–308 (1951).

GRAIN STORAGE STUDIES

XXX. Chitin Content of Wheat as an Index of Mold Contamination and Wheat Deterioration[1]

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ABSTRACT

Chitin was determined on hydrolyzed crude fiber preparations from nine wheat samples with varying degrees of mold infestation by the colorimetric procedure of Elson and Morgan and a modification of Tracey's alkaline decomposition method. Both gave the same relative results, but the latter method gave higher values with a higher experimental error. The mean chitin values (expressed as glucosamine) for the two methods varied from 18 to 393 p.p.m. dry basis for the different samples. Wheats of low mold count, low fat acidity, and high viability gave glucosamine values of 18 to 55 p.p.m. Samples with mold counts of 8.8 and 400 million spores per g. with high fat acidity and zero viability contained 214 and 393 p.p.m. glucosamine. The chitin content of four samples from a previous storage experiment was more indicative of their fat acidity and viability values than mold spore count.

The determination of chitin content offers promise in evaluating incipient grain deterioration, but for routine use the analytical procedures would have to be simplified and their replicability improved.

Molds are a major factor in the deterioration of bulk grain in storage, and the extent of mold invasion is now frequently employed as one of the criteria for detecting and evaluating the extent of spoilage. Christensen and his associates (3,4,5) have developed microbiological procedures for measuring the external and internal inoculum as well as the number of seeds deeply invaded by molds. These methods all depend upon the production of spores and do not provide a direct measure of the abundance of mycelium. A method which takes into account nonviable spores as well as living, dormant, and dead mycelium would perhaps give a more accurate picture of the extent of mold infestation. A quantitative chemical method for determining total mold mycelium would be particularly valuable for laboratories which do not have facilities or personnel for carrying out microbiological assays.

Most fungi have thick chitinous cell walls, whereas none of the vascular plants have been found to synthesize chitin (14). The mycelium of molds has been reported to contain from 3 to 5.5% chitin on a dry-matter basis (7,12). Although the amount of chitin in mold my-

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celium varies appreciably, its determination might serve as a useful chemical index of the extent of mold contamination in grain. This paper reports exploratory studies on the chitin content of wheat in relation to mold count, viability, and fat acidity.

Materials and Methods

Nine hard red spring wheat samples with varying degrees of mold infestation, as listed in Table I, were employed. Four samples of wheat from a previous storage study were included; three of these did not contain any viable mold spores, but fat acidity and viability tests indicated varying degrees of deterioration.

Separation of Chitin. Chitin has been quantitatively recovered from crawfish waste, rock lobster, and Penicillium molds by a procedure similar to that employed in obtaining the crude fiber in the proximate analysis of feeds (2,12). Successive digestion with acid and alkali concentrates the chitin and eliminates substances which would tend to interfere with the final assay.

TABLE I

MEAN CHITIN CONTENT OF WHEAT SAMPLES BY EACH OF THE TWO METHODS

Sample No.	Variety	Treatment [a]	Chitin Content as Glucosamine [b]	
			Elson and Morgan Method	Tracey Method
			ppm	*ppm*
1	Selkirk	none	32	39
2	Marquis	none, control for samples 8 and 9	15	21
3	Lee	none	48	62
4	Redman	surface-sterilized, stored 8 months at 24°C.	84	102
5	Redman	surface-sterilized, stored 8 months at 38°C.	59	72
6	Redman	mold-inoculated, stored 8 months at 38°C.	62	76
7	Redman	mold-inoculated, stored 12 months at 24°C.	75	98
8	Marquis	stored at about 25% moisture for 30 days at 24°C.	384	402
9	Marquis	stored at about 25% moisture for 30 days at 24°C.	200	227
		Mean	106.4	122.2
		Standard error (single determination)	7.1	10.1

[a] The Redman samples were either surface-sterilized or mold-inoculated as specified, conditioned to 18% moisture, and held for 7 days at 30°C., then dried to about 12% moisture and stored as indicated.

[b] Dry matter basis; each value is the mean of three determinations.

Summary of Variance Analysis

Variation due to	*DF*	*Mean Square*
Differences between methods, M	1	84058.7**
Differences between samples, S	8	3329.2**
Interaction, methods by samples, M×S	8	70.2
Experimental error	36	86.8

The wheat samples were ground in a micro Wiley mill to pass a 40-mesh sieve. Ten grams of the ground sample were extracted with ethyl ether to remove most of the lipids. The residue was refluxed with 1.0 liter of 1.25% by weight of sulfuric acid solution for 30 minutes, filtered through cloth, and washed free from acid. The residue was then refluxed with 1.0 liter of 1.25% by weight of sodium hydroxide solution according to the regular procedure for crude fiber (1). The residue was filtered and washed with several portions of hot water, followed by ethanol and ethyl ether to remove soluble coloring matter.

Methods of Assaying Crude Fiber for Chitin. According to Tracey (15), there are no quantitative methods for determining chitin which are beyond reproach. The most accurate assays are obtained by hydrolyzing the insoluble chitin[4] residue freed from fats, proteins, and soluble carbohydrates and then determining either the acetic acid or glucosamine content. Preliminary trials led to the use of the Elson and Morgan (6) and the Tracey alkaline decomposition method (14) for determining the chitin content of the "crude fiber." The Elson and Morgan method is based on the condensation of glucosamine with alkaline acetyl acetone, followed by reaction of the substituted pyrrole condensation product with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) to form a red-colored compound which is estimated colorimetrically. The Tracey method is based on the fact that glucosamine nitrogen is liberated as ammonia upon heating with trisodium phosphate.

The crude fiber from the wheat sample was placed in a hydrolysis tube and 2 ml. of 12*N* hydrochloric acid solution added. When most of the residue had dissolved, 2 ml. of distilled water were added, and the tubes were sealed and maintained in a boiling-water bath for 6 hours. The reaction mixture was filtered into a 10-ml. volumetric flask, and the filtrate was neutralized with sodium hydroxide solution and made up to 10 ml. Aliquots or appropriate dilutions were then assayed for glucosamine by the two methods.

For the Elson and Morgan method, the modification described by Südhof and Petrovic was used (13). Five milliliters of the glucosamine solution were placed in 50-ml. glass-stoppered hydrolysis tubes and treated with 2 ml. of 1.96% acetylacetone in 0.5*N* sodium carbonate solution. The tubes were tightly stoppered and placed in a boiling-water bath for 20 minutes. After cooling under tapwater, 12 ml. of 95% ethanol and 2 ml. of Ehrlich's reagent (1.6 g. of *p*-dimethylaminobenzaldehyde dissolved in 60 ml. of 95% ethanol and

[4] Chitin is composed of acetylated glucosamine units joined by beta-1,4- linkages to form long chains.

60 ml. of 12*N* hydrochloric acid) were added. The solutions were allowed to stand 45 minutes and their absorbance was read in a Coleman Junior spectrophotometer at 535 mμ after the instrument was set to zero absorbance with a reagent blank carried through with the samples. The standard curve prepared with known amounts of glucosamine hydrochloride showed a straight line relationship over the range of 25 to 125 γ in the 5-ml. aliquots.

In the alkaline decomposition method a micro-Kjeldahl steam distillation apparatus was substituted for the Markham still used by Tracey. A 2-ml. sample was placed in the steam distillation apparatus and washed down with 5 ml. of saturated trisodium phosphate solution saturated with sodium borate. The distillation was carried out at the rate of 3 ml. per minute and the distillate was collected in a test tube containing 1 ml. of 2% boric acid. The receiving tubes were graduated at 10 ml. and the distillation was stopped when the mark was reached. Several milliliters of concentrated sulfuric acid were added to the distilled water in the steam-producing unit in order to bind any traces of ammonia in the water. Blanks and standard solutions of glucosamine hydrochloride were also carried through the distillation. To each tube 1 ml. of Nessler's reagent was added and after 30 minutes' standing the absorbance was read at 490 mμ in a Coleman Junior spectrophotometer. A standard curve prepared with known amounts of glucosamine hydrochloride showed a straight-line relationship over the range of 30 to 280 γ ammonia per ml.

Other Analytical Procedures. Mold counts were made according to the method described by Christensen (4) for determining external infection of seeds.

Viability was determined by placing 50 seeds on moist blotting paper in a Petri dish maintained at 20°C. and counting the seeds which germinated after 7 days.

Fat acidity tests were performed on the diethyl ether extract of wheat according to the procedure of Hunter *et al.* (9). The fat acidity was expressed as mg. of potassium hydroxide required to neutralize the ether extract obtainable from 100 g. of wheat on a dry weight basis.

Results and Discussion

The mean results of determinations of chitin content made in triplicate on subsamples of each wheat by the two methods are recorded in Table I, together with a variance analysis of the data. The modified Tracey procedure gave significantly higher values than the method of Elson and Morgan and a significantly higher experimental error. The

TABLE II

RELATION BETWEEN FAT ACIDITY, CHITIN CONTENT, MOLD COUNT, AND VIABILITY OF THE WHEAT SAMPLES

SAMPLE NO.	VARIETY	TREATMENT[a]	FAT ACIDITY[b]	MEAN CHITIN CONTENT[c]	MOLD COUNT[d]	VIABILITY
				ppm	*thousands*	%
1	Selkirk	none	17	36	5	96
2	Marquis	none; control for samples 8 and 9	18	18	1	92
3	Lee	none	22	55	18	95
4	Redman	surface-sterilized, stored 8 months at 24°C.	39	93	0	92
5	Redman	surface-sterilized, stored 8 months at 38°C.	62	66	0	3
6	Redman	mold-inoculated, stored 8 months at 38°C.	79	69	0	30
7	Redman	mold-inoculated, stored 12 months at 24°C.	92	86	2.8	61
8	Marquis	stored at about 25% moisture for 30 days at 24°C.	104	393	400,000	0
9	Marquis	stored at about 25% moisture for 30 days at 24°C.	129	214	8,800	0

[a] The Redman samples were either surface-sterilized or mold-inoculated as specified, conditioned to 18% moisture, and held for 7 days at 30°C., then dried to about 12% moisture and stored as indicated.

[b] Fat acidity is expressed as mg. potassium hydroxide required to neutralize the free fatty acids in the diethyl ether extract per 100 g. wheat (dry basis).

[c] Expressed as glucosamine, dry matter basis. Each value is the mean of six determinations, three by the Elson Morgan and three by the modified Tracey procedures.

[d] Mold count per g. wheat.

pooled standard errors for the nine samples of 10.1 and 7.1 p.p.m. respectively are 8.3 and 6.7% of the corresponding means. These percentage errors are considerably higher than those for protein and are somewhat higher than those for thiamine assays made on the same series of samples in different laboratories (10,11). This high relative error would be expected, since it includes those involved in the isolation of the crude fiber, hydrolysis of the chitin, and the assay of glucosamine. The two methods give the same relative results for the various samples, as the interaction, methods × samples, is not statistically significant.

The mean chitin values by the two procedures are summarized in Table II, together with the mold count, viability, and fat acidity of the wheat samples. The chitin contents of the heavily mold-infested samples (8 and 9) were markedly higher than those of the samples of sound wheat (1, 2, and 3) of high viability which had not been subjected to any special storage treatment. While the chitin contents of samples 8 and 9 place them in the same order as mold count, they are not in relative agreement with the fat acidity values. However, in a

previous study it was found that fat acidity sometimes decreased as mold growth became extensive, indicating the utilization of fatty acids by the fungi (3). The chitin values for the Redman samples from a previous storage experiment (8) are of particular interest. The prestorage treatment of the samples by either surface-sterilization or mold-inoculation, followed by holding the samples at 18% moisture for 7 days at 30°C., would permit viable molds to grow. After air-drying at room temperature (approx. 24°C.) to about 12% moisture, samples 4, 5, 6, and 7 gave mold counts of 0, 0, 31,000, and 31,000 respectively. During subsequent storage at 12% moisture the mold counts of samples 6 and 7 fell to 0 and 2,800 respectively. In this particular series the mold counts alone did not reflect accurately the storage condition of these samples. The chitin values, however, are in line with the fat acidity and viability data.

These limited experiments indicate that the chitin determination might be useful as a criterion for evaluating the extent of deterioration of wheat. Although the results indicate that the degree of mold infestation occurring in wheat is amenable to chemical analysis, the determination of chitin would have to be simplified and its replicability improved before it would be of any value in routine work.

Acknowledgment

The authors are indebted to C. M. Christensen, Department of Plant Pathology and Botany, University of Minnesota, for determining the mold counts.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. Black, M. M., and Schwartz, H. M. The estimation of chitin and chitin nitrogen in crawfish waste and derived products. Analyst **75**: 185–189 (1950).
3. Bottomley, R. A., Christensen, C. M., and Geddes, W. F. Grain storage studies. X. The influence of aeration, time, and moisture content on fat acidity, nonreducing sugars, and mold flora of stored yellow corn. Cereal Chem. **29**: 53–64 (1952).
4. Christensen, C. M. Fungi on and in wheat seed. Cereal Chem. **28**: 408–415 (1951).
5. Christensen, C. M., and Drescher, R. F. Grain storage studies. XIV. Changes in moisture content, germination percentage, and moldiness of wheat samples stored in different portions of bulk wheat in commercial bins. Cereal Chem. **31**: 206–216 (1954).
6. Elson, L. A., and Morgan, W. T. J. A colorimetric method for the determination of glucosamine and chondrosamine. Biochem. J. **27**: 1824–1828 (1933).
7. Foster, J. W. Chemical activities of fungi, pp. 90–96. Academic Press: New York (1949).
8. Golubchuk, M., Sorger-Domenigg, H., Cuendet, L. S., Christensen, C. M., and Geddes, W. F. Grain storage studies. XIX. Influence of mold infestation and temperature on the deterioration of wheat during storage at approximately 12% moisture. Cereal Chem. **33**: 45–52 (1956).
9. Hunter, I. R., Houston, D. F., and Kester, E. B. Development of free fatty acids during storage of brown (husked) rice. Cereal Chem. **28**: 232–239 (1951).
10. Meredith, W. O. S. Analysis of data for AACC Check Sample Service. I. Protein

and thiamine results 1943–44. Cereal Chem. 22: 437–448 (1945).
11. MEREDITH, W. O. S. Analysis of data for AACC Check Sample Service. II. Protein and thiamine results 1944–45. Cereal Chem. 23: 585–590 (1946).
12. SMITHIES, W. R. Chemical composition of a sample of mycelium of *Penicillium griseofulvum* Dierck. Biochem. J. 51: 259–264 (1952).
13. SÜDHOF, H., and PETROVIC, C. Zur Glucosaminbestimmung im Serum. Z. physiol. Chem. 298: 70–72 (1954).
14. TRACEY, M. V. The determination of glucosamine by alkaline decomposition. Biochem. J. 52: 265–267 (1952).
15. TRACEY, M. V. Chitin. *In* Modern methods of plant analysis, vol. II; ed. by K. Palch and M. V. Tracey. Springer-Verlag: Berlin (1955).

A NOTE ON AN IMPROVED DENATURATION TEST FOR GLUTEN BASED ON SOLUBILITY IN ACETIC ACID[1]

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R. J. DIMLER, and F. R. SENTI

A number of modifications are proposed to extend the usefulness of the solubility test described by Blish (2) for evaluating the extent of denaturation of wheat gluten. The modifications consist in lowering the concentrations of gluten and of acetic acid, eliminating the use of alcohol, and substituting vigorous stirring for overnight standing and also centrifugation for filtration. These changes have increased the speed, simplicity, and accuracy of the test.

The modified method is: Disperse 0.320 g. (dry basis) of gluten in 100 ml. of 0.01*N* acetic acid by agitation in a Waring Blendor[2] for 3 minutes. Centrifuge at 3300 × G for 30 minutes. Determine the percent of total nitrogen remaining in supernatant by the Kjeldahl procedure.

This method provides a greater solubility differential between undenatured and extensively denatured gluten than does the Blish method, largely because the undenatured gluten is more soluble. Typical values for percent of soluble nitrogen are as follows:

	Blish Method	*Improved Method*
Undenatured gluten	76	89
Gluten heated at 90°C. for 30 minutes	7	5

Another advantage of the present method is that it gives reliable results with lyophilized glutens. For example, when a vacuum-dried gluten was dispersed in 0.01*N* acetic acid and lyophilized, its solubility

[1] Manuscript received September 11, 1959. Contribution from Northern Regional Research Laboratory, Peoria, Illinois. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

[2] Mention of firm names or trade products does not constitute endorsement or rejection by the U.S. Department of Agriculture over others of a similar nature not mentioned.

by our method was 89% before and 91% after lyophilization. These values are in keeping with literature reports that lyophilization does not denature gluten (1,3). In contrast, when determined by the Blish method, the solubility dropped from 76% before to 58% after lyophilization. Presumably this decrease in solubility resulted from partial denaturation of lyophilized gluten by the alcohol used as a wetting agent.

The response of solubility to increasing extents of denaturation is illustrated by results obtained with laboratory-prepared gluten heated in water:

Treatment	*Soluble N*
	%
Unheated, not dried	92
60°C., 15 minutes, not dried	93
80°C., 15 minutes, not dried	83
95°C., 5 minutes, not dried	50
95°C., 15 minutes, not dried	21
95°C., 15 minutes, dried at 48°C.	9
95°C., 30 minutes, dried at 48°C.	2

Commercially prepared vital glutens gave values between 84 and 93% soluble nitrogen. This range may reflect differences either in the original wheats or in the extent of denaturation during manufacture.

Acknowledgment

The authors are indebted to R. A. Anderson of this Division for supplying some of the gluten samples which were tested.

Literature Cited

1. Adams, G. A. New laboratory methods for drying wheat gluten. Cereal Chem. 29: 312–314 (1952).
2. Blish, M. J. Wheat gluten. *In* Advances in protein chemistry, ed. by M. L. Anson and J. T. Edsall, vol. 2, pp. 337–359. Academic Press: New York (1945).
3. Lusena, C. V. Preparation of dried native wheat gluten. Cereal Chem. 27: 167–168 (1950).

ERRATA

Cereal Chemistry, Vol. 36, No. 6
(November, 1959)

Page 486, Rezsoe Gracza

Literature reference 9: delete second line and substitute:
powders. An air-permeation apparatus. Ind. Eng. Chem., Anal. Ed. 12: 479–482 (1940).

Pages 522 and 528, Lee, Cuendet, and Geddes

Page 522, Abstract, paragraph 2:

Delete second sentence, reading "Doughs with no added sugar . . . sucrose."

In third sentence, delete the word "following", to read: "In the 60-minute panproof period . . ."

Page 528:

Table II, last column, change Maltose value to 500; Mean, to 499; Standard error, to 28.6; Coefficient of variability (%), to 5.7.

Delete paragraph 3 reading "During the first 40 minutes . . . glucose and fructose."

In paragraph 4, delete the second sentence reading "Those made with glucose . . . containing maltose."

Cereal Chemistry

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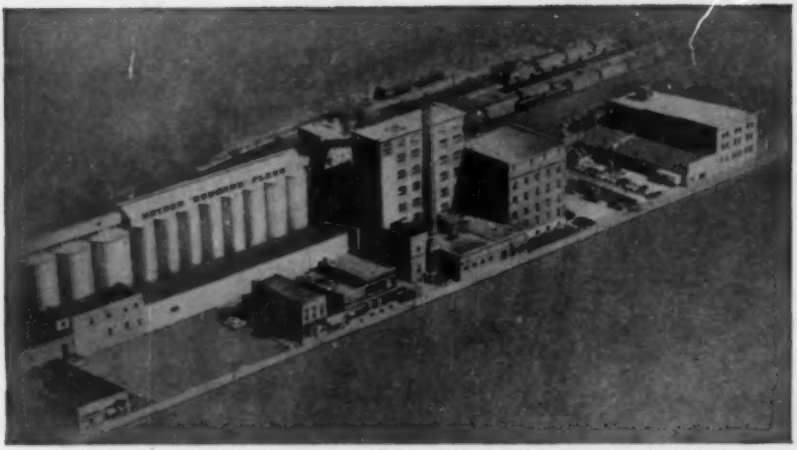

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